FACULTY OF PHARMACEUTICAL SCIENCES

Testing the activity of novel psychedelics on the 5-HT_{2A} and 5-HT_{2B} receptor

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SUMMARY

Over the last 10 years a shift in recreational drug use has occurred from well-known illicit drugs to novel psychoactive substances (NPS). Synthetic hallucinogens are one of the emerging classes within the NPS. They exert their effect through the stimulation of serotonin receptors in the brain, with the serotonin receptor (5-HTR) 2A (5-HT_{2A}R) being the main mediator of hallucinogenic effects. However, the contribution of other receptors such as the 5-HT_{1A}R, 5-HT_{2B}R and 5-HT_{2C}R to hallucinogenic effects and possible side effects should not be forgotten. The hallucinogens can be divided into two main categories: phenylalkylamines and indoleamines. The indoleamines can be further classified into lysergamides and tryptamines. In this dissertation the activity (i.e., efficacy and potency) of 5 tryptamine derivatives 4-AcO DMT, 4-AcO MPT, 5-MeO MiPT, 5-MeO AMT and 5-chloro tryptamine will be determined on the 5-HT_{2A}R and 5-HT_{2B}R using the AequoScreen Assay. Since the tested tryptamine derivatives were found on the illicit market and are not yet regulated in Canada, this thesis aims to get insight into their activity and potential risks. Based on that, a decision can be made regarding the regulation of these substances.

The AequoScreen Assay is based on measuring a chemiluminescent signal caused by Ca^{2+} flux. An increase in Ca^{2+} flux in the cytosol occurs when the 5-HT_{2A}R or 5-HT_{2B}R is activated. From the data, a three-parameter, non-linear regression log(concentration)-response curve can be constructed. The efficacy (E_{max}) and potency (EC_{50}) can be derived.

4-AcO DMT was a partial agonist on the 5-HT_{2B}R and a strong (>80%) partial agonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and the 5-HT_{2A}R was 969.9 nM and 2021 nM respectively. 4-AcO MPT was a partial agonist on the 5-HT_{2B}R and a weak (<20%) partial agonist or even antagonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and 5-HT_{2A}R was 577.9 nM and 29.64 nM. 5-MeO MiPT was a partial agonist on the 5-HT_{2B}R and a full agonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and 5-HT_{2A}R was 11.40 nM and 263.5 nM. 5-MeO AMT was a partial agonist on the 5-HT_{2B}R and a full agonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and 5-HT_{2A}R was 15.58 nM and 4.665 nM. 5-chloro tryptamine was a partial agonist on the 5-HT_{2B}R and a full agonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and 5-HT_{2A}R was 11.40 nM and 29.47 nM.

SAMENVATTING

In de afgelopen tien jaar vond een verschuiving plaats in het recreatief drugsgebruik van de bekende, illegale drugs naar nieuwe psychoactieve stoffen (NPS). De synthetische hallucinogenen vormen een van de opkomende klassen binnen de NPS. Ze oefenen hun effect uit via stimulatie van serotonine receptoren in de hersenen, waarbij de 5-HT_{2A}R de belangrijkste mediator is voor hallucinogene effecten. De bijdrage van andere receptoren zoals de 5-HT_{1A}R, 5-HT_{2B}R en 5-HT_{2C}R tot hallucinogene effecten en bijwerkingen mag ook niet worden vergeten. De hallucinogenen kunnen worden opgedeeld in twee categorieën: fenylalkylamines en indolamines. De indolamines kunnen verder worden ingedeeld in de lysergamides en de tryptamines. In deze thesis wordt de activiteit van de vijf tryptamine-derivaten 4-AcO DMT, 4-AcO MPT, 5-MeO MiPT, 5-MeO AMT en 5-chloortryptamine, bepaald ter hoogte van de 5-HT_{2A}R en 5-HT_{2B}R met behulp van de AequoScreen Assay. Aangezien de geteste tryptamine-derivaten op de illegale markt zijn gevonden en nog niet gereguleerd zijn in Canada, is het doel om inzicht te krijgen in hun activiteit en gevaar. Op basis daarvan kan worden bepaald of deze stoffen gereguleerd moeten worden.

De AequoScreen Assay is gebaseerd op het meten van een chemiluminescent signaal veroorzaakt door Ca^{2+} flux. Een toename van Ca^{2+} in het cytosol treedt op wanneer de 5-HT_{2A}R of 5-HT_{2B}R geactiveerd wordt. Op basis van de gemeten waarden kan een drie-parameter, niet-lineaire regressie log(concentratie)-responscurve worden geconstrueerd.

4-AcO DMT was een partiële agonist op de 5-HT_{2B}R en een sterke (>80%) partiële agonist op de 5-HT_{2A}R. De potentie op de 5-HT_{2B}R en de 5-HT_{2A}R was respectievelijk 969,9 nM en 2021 nM. 4-AcO MPT was een partiële agonist op de 5-HT_{2B}R en een zwakke (<20%) partiële agonist of zelfs antagonist op de 5-HT_{2A}R. De potentie op de 5-HT_{2B}R en 5-HT_{2A}R was 577,9 nM en 29,64 nM. 5-MeO MiPT was een partiële agonist op de 5-HT_{2B}R en een agonist op de 5-HT_{2A}R. De potentie op de 5-HT_{2B}R en 5-HT_{2A}R was 11,40 nM en 263,5 nM. 5-MeO AMT was een partiële agonist op de 5-HT_{2B}R en een agonist op de 5-HT_{2A}R. De potentie op de 5-HT_{2B}R en 5-HT_{2A}R was 15,58 nM en 4,665 nM. 5-chloortryptamine was een partiële agonist op de 5-HT_{2B}R en een agonist op de 5-HT_{2A}R. De potentie op de 5-HT_{2B}R en 5-HT_{2A}R was 11,40 nM en 29,47 nM.

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TABLE OF CONTENTS

ABBREVIATIONS

4-AcO DMT	4-acetoxy-N,N-dimethyltryptamine
4-AcO MPT	4-acetoxy-N-methyl-N-propyltryptamine
5-HT	serotonin
5-HT_{1A}R	serotonin 1A receptor
5-HT_{2A}R	serotonin 2A receptor
5-HT_{2B}R	serotonin 2B receptor
5-HT_{2C}R	serotonin 2C receptor
5-MeO AMT	5-methoxy-α-methyltryptamine
5-MeO MiPT	5-methoxy-N-methyl-N-isopropyltryptamine
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
CHO-K1	Chinese Hamster Ovary -K1 cells
CME	clathrin-mediated endocytosis
CNS	central nervous system
DAG	diacylglycerol
DMEM/F-12 medium	Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12
DMSO	dimethyl sulfoxide
DMT	N,N-dimethyltryptamine
EU	European Union
FBS	foetal bovine serum
FLIPR	fluorescence imaging plate reader
GDP	guanosine diphosphate
GEF	guanine nucleotide exchange factor
GPCR	G protein coupled receptor
GRK	GPCR kinase
GTP	guanosine triphosphate
h5-HT_{2A}R	human serotonin 2A receptor
h5-HT_{2B}R	human serotonin 2B receptor
IP_3	inositol triphosphate

LSD	lysergic acid diethylamide
MDMA	methylenedioxy-methylamphetamine
MOA	monoamine oxidase
NPS	Novel Psychoactive Substance(s)
PBS	phosphate buffer saline
PIP_2	phosphatidylinositol-4,5-biphosphate
PKC	protein kinase C
PLCβ	phospholipase C β
RD	Royal Decree
SD	standard deviation
SEM	standard error of the mean
SSRI	selective serotonin reuptake inhibitors

1 INTRODUCTION

1.1 ANATOMICAL STRUCTURE OF THE 5-HT RECEPTOR

In the human body, 7 different sub-types of serotonin receptors (5-HT_{1-7}) have been identified. Six out of the 7 sub-types can be classified as G protein coupled receptors (GPCR). (1) The 5-HT_3 receptor is the only sub-type that is not a GPCR, this receptor functions as a ligand-gated ion channel. The 5-HT_3 receptor can be distinguished from the other families, as the structure of the receptor and its functionality differ from the other sub-types. This receptor type binds with a ligand e.g., the neurotransmitter serotonin (5-HT) which results in the rapid opening of the coupled ion channel (Na^+/K^+ channel) resulting in cell membrane depolarization. (2)

The other 6 sub-types of serotonin receptors are known to be class A (i.e., rhodopsin-like receptors) GPCRs. A ligand binds to the ligand-binding pocket in the 7 transmembrane (7TM) regions of the GPCR. Class B is known as the secretin family and binds its ligand using an extracellular loop and 7TM domains. GPCRs that are class C are part of the metabotropic glutamate receptors and binds a ligand in a ligand-binding pocket in the extracellular domain with a Venus flytrap. The first three classes of the GPCRs are the most common and the best known. Class D - fungal mating pheromone receptors, class E - cAMP receptors and class F - frizzled and smoothened receptors are the final three classes of GPCR's. (3) The class A GPCRs contain 7 transmembrane regions, have 3 extracellular loops and 3 intracellular loops. The receptors are peptides with the amino terminus extracellular and the carboxyl terminus intracellular. The activation of the GPCR (i.e., binding of an agonist) produces a conformational change in the receptor that promotes the binding of a G protein to the intracellular part of the receptor which is then able to exchange guanosine diphosphate (GDP) for guanosine triphosphate (GTP) and modulate secondary messenger molecule signalling cascades. (Figure 1.1). (4)

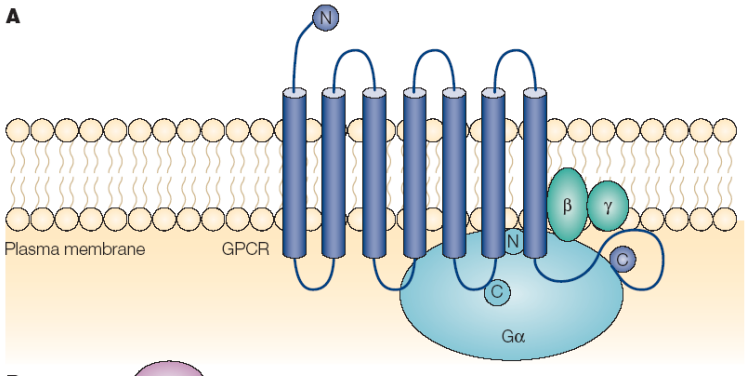

Figure 1.1: General structure of a G protein coupled class A receptor consisting of 7 transmembrane regions, 3 extracellular loops, 3 intracellular loops and coupled to a heterotrimeric G protein when activated. Image used from (5)

1.2 SINGALLING OF A GPCR

1.2.1 Interaction of GPCR with a G protein

The G proteins are heterotrimeric complexes consisting of a monomeric Gα subunit and Gβγ dimeric subunit, with Gα bound to GDP when in an inactive state. After binding an agonist to the receptor a G protein binds the intracellular surface of the GPCR and GDP dissociates from Gα while GTP binds to Gα. The exchange is catalysed by guanine nucleotide exchange factors (GEFs). (6) This causes a conformational change of the heterotrimeric complex where the heterotrimeric G protein diffuses from the GPCR and Gα diffuses from Gβγ. The Gα monomer interacts with an effector protein, which can be activated or inhibited. In some cases, the Gβγ dimer interacts with an effector protein (Figure 1.2). The G protein activation is terminated by hydrolysis of GTP to GDP and Pi. (5)

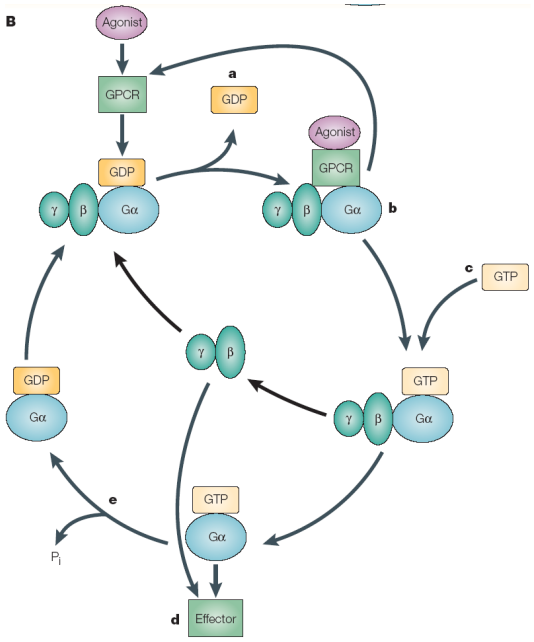

Figure 1.2: The result of binding of an agonist to the GPCR. The heterotrimeric G protein binds to the GPCR. Upon binding GDP dissociates from Gα, GTP binds to Gα and Gα diffuses from Gβγ. Gα or Gβγ interacts with the effector protein which causes an effect in the cell. Activation of the G protein is terminated by hydrolysis of GTP bound to Gα. Image used from (5)

The effectors of the G protein can be ion channels or enzymes. Activation or inhibition of the effectors causes the movement of ions across the plasma membrane or the interconversion of substrates, such as cyclic adenosine monophosphate (cAMP). Depending on which Gα monomer is coupled to the activated GPCR a different signalling pathway is activated. There are 4 families of α subunits: $G_{i,}$ $G_{s,}$ $G_{12/13}$ and $G_{q.}$ as shown in Figure 1.3. (6) (7)

$G\alpha_s$ (stimulatory) and $G\alpha_i$ (inhibitory) regulate adenylate cyclase (AC). AC binds to Gα which stimulates or inhibits the conversion of adenosine triphosphate (ATP) to cAMP. Next to that, $G\alpha_i$ also has an influence on the K^+ concentration in the cell via Gβγ. $G\alpha_q$ activates phospholipase Cβ (PLCβ). PLCβ hydrolyses phosphatidylinositol-4,5-biphosphate (PIP_2) to second messengers 1,4,5-inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 opens Ca^{2+} channels in the endoplasmatic reticulum of the activated cell and causes the release of Ca^{2+} in the cytosol. DAG activates protein kinase C. Activation of $G_{12/13}$ causes Rho activation using GEFs for small GTPases of the Rho family. (1) (6) (8) (9) Depending on which serotonin receptor will be activated, a different Gα will be coupled and a different effector protein will be activated or inhibited.

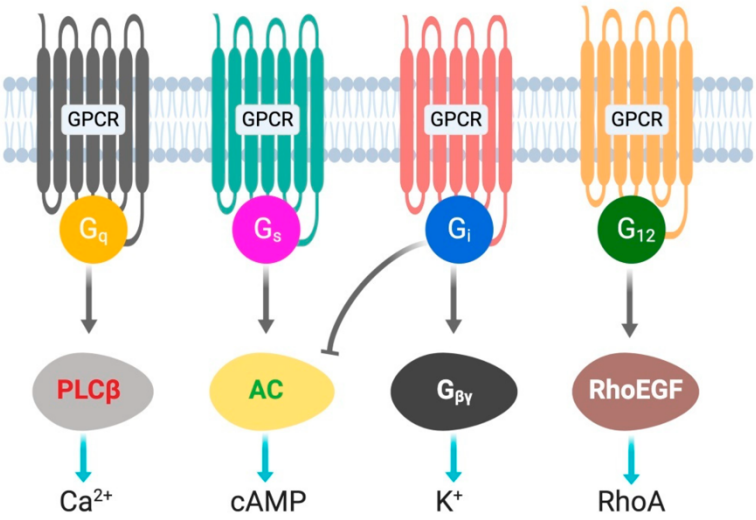

Figure 1.3: G protein classification and downstream signals. $G\alpha_q$ activates PLCβ, which causes the formation of IP_3 and DAG, which opens the Ca^{2+} ion channels in the endoplasmatic reticulum in the cell and activate PKC respectively. $G\alpha_s$ (stimulatory) and $G\alpha_i$ (inhibitory) regulate AC, next to that $G\alpha_i$ also influence the K^+ concentration in the cell. Activation of $G_{12/13}$ causes Rho activation. Image used from (9).

1.2.2 Interaction of GPCR with β-arrestin

Termination of signalling is a two-step process. First, amino acids in the intracellular loops of the GPCR are phosphorylated by specific receptor kinase i.e., GPCR kinase (GRK). Phosphorylation of GPCRs does not cause a complete blockade of the G protein dependent signalling. A complete blockade of the G protein dependent signalling requires arrestin binding to the receptor. Recruitment of cystolic arrestin proteins happens following GRK phosphorylation of the GPCR. There are 4 different classes of arrestin proteins (arrestin 1 – 4). The non-visual arrestins or arrestin 2 and arrestin 3 are the best known and expressed in most cells. They are also called β-arrestin1 and β-arrestin2 respectively. (10) (11) There is a competition between the G-protein and β-arrestin for binding to the GPCR. When the GPCR is phosphorylated, it has a high affinity for β-arrestin and the β-arrestin can easily outcompete the G-protein. After binding to the GPCR, β-arrestin sterically hinders the interaction of a G-protein and the GPCR. Therefore, G protein-mediated signalling terminates or receptor desensitization happens. (7)

β-arrestin plays an important role in receptor desensitization, but next to that β-arrestin also has other functions (Figure 1.4). It plays a part in the internalization of GPCRs. After phosphorylation and binding of β-arrestin to the GPCR, the GPCR interacts with proteins of the clathrin-coated pits machinery and clathrin-mediated endocytosis (CME) occurs. (12) After internalization the GPCRs are sorted into receptors for degradation and receptors for recycling. This process is called receptor trafficking. (13) Receptors that are marked for degradation will be degraded enzymatically at the lysosome and receptors marked for recycling will be dephosphorylated and transferred back to the plasma membrane. Lastly, β-arrestin is also important in the signal transduction of the cell and can activate G protein independent pathways such as the activation of different kinase pathways (e.g., MAPK, ERK1/2, etc.). (14)

An apparent phenomenon that is seen at the 5-HT receptors is “biased signalling” or “functional selectivity”. Most endogenous agonists (e.g., 5-HT) when activating a 5-HTR, are able to both activate the G protein signalling pathway and the β-arrestin pathway. On the contrary, some 5-HTR agonists (e.g., LSD and ergotamine) have been suggested by some authors to prefer one signalling pathway over the other. (15) By binding of that agonist to the receptor, the agonist stabilises a specific receptor conformation. Based upon that conformation, a specific signal transduction pathway is activated. (16) (17) (18) By reading the literature on this topic, it is clear that there is no consensus on this matter with regard to which agonists are “biased” or what the functional implication of those biases may be in whole living organisms. (19) (20) (21) Further investigation into biased signalling and biased agonists is needed.

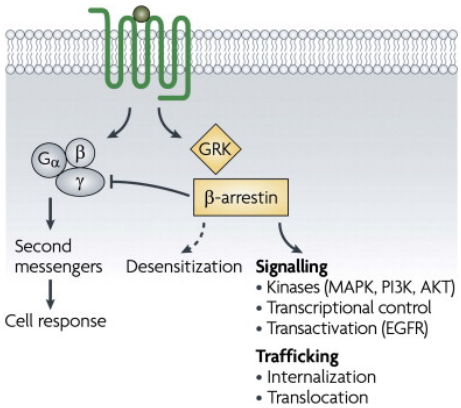

Figure 1.4: GPCR signalling via the G protein mediated pathway and the β-arrestin mediated pathway. Image used from (13).

1.3 SIGNALLING OF A 5-HT RECEPTOR

5-HT receptors can be classified in different families. In each family a further subdivision happens according to genetic homology. In total there are 14 receptor subtypes known. The focus of this thesis will lie on the class of the 5-HT_2 receptors, especially the 5-HT_{2A} and 5-HT_{2B} receptors. The third member of this class is the 5-HT_{2C} receptor. These receptors were grouped due to their high structural homology. All 3 subtypes in the 5-HT_2 receptor family are class A GPCRs and signalling happens preferentially through the $G\alpha_q$ signalling pathway. Activation of the receptor by an agonist, causes a conformational change of the receptor. The heterotrimeric G protein can bind the receptor intracellularly and eventually $G\alpha_q$ activates phospholipase C (PLC), which causes the conversion of PIP_2 to IP_3 and DAG. (1) (6) (22) The effects caused by the second messenger is described in paragraph 1.2.1.

1.3.1 5-HT_{2A} receptor

The 5-HT_{2A} receptor (5-HT_{2A}R) is known to be the receptor responsible for the hallucinogenic and neuropsychological effects of serotonergic psychedelics. (23) (24) These effects are caused by the 5-HT_{2A}R in the brain. The 5-HT_{2A}R are found throughout different regions of the brain but are more abundant in certain, specific regions. After carefully reviewing numerous articles, it appears there is a lack of consensus describing the presence of the 5-HT_{2A}R in specific brain regions. Generally, the 5-HT_{2A}R is most-abundant in the cerebral cortex and the nucleus caudatus. 5-HT_{2A}R can also be found in the thalamus and the hypothalamus but here they are less densely distributed. Lastly, they can also be found in the blood vessels of the brain. (8)

The 5-HT_{2A}R is also present outside of the CNS, in the periphery of the body. The 5-HT_{2A}R have a function in the cardiovascular system since the receptors are prevalent on the smooth muscles in the blood vessels and the platelets. When an agonist (e.g., 5-HT) binds to the receptors on the smooth muscle, they influence vascular tone and blood pressure. By binding the receptors on the platelets, they induce platelet aggregation. (25) (26) (27) Stimulation of the 5-HT_{2A}R also causes an increase in vascular permeability. (26) Next to their presence in the blood vessels and bloodstream, the 5-HT_{2A}R can also be found in the heart where they enhance cardiac contractility. (27)

Secondly, 5-HT_{2A}R can also be found in the urogenital system where they are most abundant in the lower urinary tract. Stimulation of these receptors causes a contraction in the lower urinary tract. (28) The 5-HT_{2A}R is also present in the uterus where they stimulate contraction of the uterine smooth muscle. (26)

A last example is the presence of the 5-HT_{2A}R in the pulmonary system, more specifically in the smooth muscles in the airway. Activation of these receptors by an agonist (e.g., 5-HT) causes a bronchoconstriction. (26)

5-HT_{2A}R has a very wide distribution through the human body. By stimulating or inhibiting the receptors a lot of different effects may occur. The focus in this thesis will be determining the G protein-dependent effects in vitro, caused by receptors that would occur in the brain. By considering the wide spread of receports a lot of side effects of the hallucinogens can be explained.

1.3.2 5-HT_{2B} receptor

The 5-HT_{2B} receptor (5-HT_{2B}R) used to be known as the 5-HT_{2F} receptor since it was characterized and cloned for the first time in the stomach fundus of a rat. Later the 5-HT_{2F}R was renamed to the 5-HT_{2B}R using the official nomenclature scheme. (29)

The focus of this dissertation will be to estimate the G protein-dependent effects by determining the activity on the 5-HT_{2A}R and 5-HT_{2B}R. As said before (paragraph 1.3.1), the hallucinogenic effect of a drug is caused by activity on the 5-HT_{2A}R mainly, but psychedelics also have an activity on the 5-HT_{2B}R which can also be found in specific brain regions. (22) Studying the presence of 5-HT_{2B}R in specific brain regions is more difficult than studying the presence of 5-HT_{2A}R in specific brain regions. This is due to a lack of agents with selectivity for the 5-HT_{2B}R. (30) The 5-HT_{2B}R is abundant in the frontal cortex, the dorsal hypothalamus, the medial ganglia, the cerebellum and the lateral septum. (30) (31) Again, there is a lack of consensus describing the presence of 5-HT_{2B}R in specific brain regions.

5-HT_{2B}R is present in the CNS but can also be found in different tissues outside of the central nervous system. A first example is the presence of the 5-HT_{2B}R in the heart.

The binding of a 5-HT_{2B}R agonist (e.g., fenfluramine) can lead to the development of severe valvulopathies. (22) (30) The use of a 5-HT_{2B}R agonist has to be done with care to avoid severe valve damage. (15)

A second example was mentioned previously, namely, the presence of the receptor in the stomach fundus, which is the upper part of the stomach next to the cardia and the body. (32) It functions as storage space for the gasses being formed during digestion. Most of the time the fundus does not store any food, but it can store food when the stomach gets full. (32) Activation of 5-HT_{2B}R in the fundus causes fundal contraction.

Another example is the presence of 5-HT_{2B}R in the pancreas. Stimulation of the β-cells of the pancreas causes the secretion of insulin and 5-HT. 5-HT plays an important role in maintaining glucose homeostasis. Secretion of 5-HT from the β-cells of the pancreas, causes an interaction between serotonin and the 5-HT_{2B}R. This interaction stimulates β-cell proliferation. After the proliferation of the β-cells, there are more cells available that can be triggered and an increase in insulin secretion can be observed. (33) (34)

The 5-HT_{2B}R is present in a lot of parts of the human body. The 5-HT_{2B}R can be found in the brain, heart, fundus, pancreas, lung, gut, kidney, etc. Therefore, activation of the receptor can cause an arsenal of effects on the body in addition to well-known hallucinatory effects. (35)

1.3.3 Other serotonin receptors responsible for hallucinogenic effects

The main mediator of hallucinogenic effects caused by psychedelics is the 5-HT_{2A}R. Next to these receptors, the impact of other 5-HTR as 5-HT_{1A}R, 5-HT_{2B}R and 5-HT_{2C}R cannot be forgotten. (23)

1.4 NOVEL PSYCHOACTIVE SUBSTANCES

The Novel Psychoactive Substances (NPS) are defined by the United Nations Office on Drugs and Crime as

"Substances of abuse, either in a pure form or a preparation, that are not controlled by the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat." (36)

This definition provides a legal framework under which we can see NPS but does not provide any insight into what NPS really are: which substances may be considered NPS, what effects NPS create, etc. NPS are a diverse group containing numerous substances often known as designer drugs, synthetic drugs, or "legal highs". They are usually either analogues of existing controlled pharmaceuticals (e.g., adding a Br^-, I^- to an existing compound) or newly synthesized substances that mimic the action and psychoactive effects of licensed drugs and other controlled substrates. (37)

An important note to make is that the word *novel* in NPS does not necessarily refer to the fact that the drugs are newly synthesized. Many of the substances classified under NPS have been available for many years but are now being misused more. The term novel thus refers to "newly misused and abused". (38) (39)

Depending on the structure and effect of the drug, it can be classified into one of the NPS groups: the synthetic stimulants, the synthetic hallucinogens, the synthetic cannabinoids, and the synthetic depressants. In each group a further subdivision can be made according to chemical structure. In this dissertation the focus will be on the synthetic hallucinogens, more specifically phenylalkylamines, lysergamides, and tryptamines. (37) (40)

1.4.1 Legislation of NPS

Legislation of the NPS can be described on an international level, a regional level for European Union (EU) countries, and a national level for each country. (41) Since the research for the dissertation was done in Saskatoon, Canada at the University of Saskatchewan and the defence will be done in Ghent, Belgium at Ghent University the legal system for both countries will be described briefly.

On September 6th 2017, a new Royal Decree (RD) in Belgium was published regarding NPS. In this new legislation changes were made in order to have better control

over NPS. Groups of psychoactive substances (amphetamines, cathinone, tryptamines, piperazines, synthetic cannabinoids and fentanyl) were described rather than specific psychoactive substances. This ensures that not only the already synthesized and available substances are covered in this RD, but new, yet-to-be synthesized drugs within these groups are covered by the RD as well. (42) (43)

Legislation of NPS in Canada is covered under the Controlled Drugs and Substances Act. This act classifies every single drug and substance into a schedule (I to IX) where the substances are being scheduled based on their medicinal value, harmfulness, and potential for abuse or dependence. (44) Since every substance must be mentioned separately in one of the schedules, they have to be kept up to date and all single, newly discovered (illicit) substances have to be included as quickly as possible. (45)

The Belgian and Canadian laws differ from each other. In Belgium a group of substances are immediately described. Every drug that is part of one of the described classes is covered by the RD. Where the Canadian law establishes a nominative list with every known drug on the (illicit) market right now. Canadian law requires the rapid identification of the newest synthesized drugs. Consequently, adaptations must be made continuously.

1.4.2 The synthetic hallucinogens

Hallucinogens or psychedelics exert their effects through stimulation of 5-HTR in the brain, where the 5-HT_{2A}R is the most important for causing the hallucinogenic effect. (24) (46) They can alter perception, mood and cognitive processes. (23) The hallucinogens can be divided into two main categories: phenylalkylamines and indoleamines. (47) The indoleamines can be further classified as lysergamides and tryptamines. (37) A lot of research has already been conducted in using hallucinogens as therapies in psychiatric settings. For example, lysergic acid diethylamide (LSD), a lysergamide, is being tested as a treatment for alcohol addictions, depression, and anxiety. (48) In addition to LSD, many other hallucinogens (e.g., mescaline, dipropyltryptamine, psilocybin etc.) are also being tested for their therapeutic value in the treatment of alcohol addictions, anxiety, and therapy-resistant depression. (49)

Hallucinogens are still most known because of the recreational misuse of these compounds. By activation of the 5-HT_{2A}R in the brain, they can cause a change in mood, sensory perception, sleep, hunger, body temperature, sexual behaviour and muscle control. (50) Hallucinogens are mostly being misused because of their ability to produce joy, euphoria, alternations in time and space perceptions, an increase in creativity, accelerating and broadening thought processes, alerted consciousness, etc. in users. (37) (49)

The hallucinogenic effects are caused by stimulation of receptors in the brain, but 5-HT_{2A}R and 5-HT_{2B}R are expressed throughout the body (peripherally and centrally) where they also have their specific effects (paragraphs 1.3.1 and 1.3.2). Stimulation of these receptors can lead to some serious side effects that we can define as serotonin syndrome. Serotonin syndrome is mediated by overstimulation of all different kinds of 5-HTR in the body with a substantial contribution of the 5-HT_{2A}R but the contribution of for example the 5-HT_{1A}R, and 5-HT_{2C}R cannot be forgotten. The 5-HT_{2C}R are partially responsible for the hunched back that can be seen in serotonin syndrome. (51) (52) (53) Next to that, overstimulation of the 5-HT_{2B}R present in the heart and cardiovascular system, can have a negative effect on the cardiovascular system. Chronic use can lead to cardiovascular complications. (15) (54)

Overstimulation can be caused by numerous drugs that influence the serotonin levels in the body (e.g., selective serotonin reuptake inhibitors (SSRI), monoamine oxides (MAO) inhibitors) or drugs that mimic the effect of serotonin. Drugs of abuse such as MDMA, LSD, 5-MeO-DiPT, etc. can overstimulate 5-HT_{2A}R when taken in excessive amounts, when combined with each other or when combined with other drugs that affect 5-HT levels in the body. (55) (56)

Serotonin syndrome can be recognized by a triad of clinical symptoms (Figure 1.5) resulting from neuroexcitation. The first group of symptoms that occurs can be described under the heading of neuromuscular hyperreactivity (hyperreflexia, clonus, myoclonus, tremor, rigidity). Second, serotonin syndrome is also characterized by autonomic hyperreactivity (hyperpyrexia, tachycardia, flushing, mydriasis, diarrhoea, and diaphoresis). Finally, it is also seen that patients with serotonin syndrome have an altered

mental status (agitation, anxiety, hypomania, confusion, and hallucinations). The manifestation and presentation of serotonin syndrome can range from barely noticeable to severe and possibly lethal. (55) (56) (51)

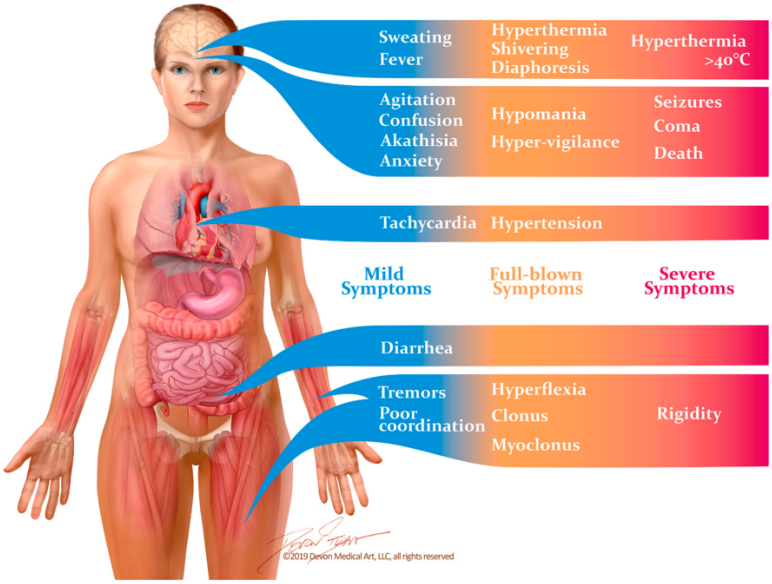

Figure 1.5: Signs and symptoms of serotonin syndrome where a subdivision is made between the mild symptoms full-blown symptoms and severe symptoms. If the mild symptoms are not recognized fast and acted upon further progression and deterioration of the patients happens which can lead to death. Image used from (56).

Diagnosis of serotonin syndrome is difficult due to the wide range of symptoms and the lack of laboratory testing that can confirm serotonin syndrome. (56) Nevertheless, serotonin toxicity can be life-threatening because of the hyperthermia caused by extreme muscle activity, an increased muscle tonus that can cause respiratory failure and secondary rhabdomyolysis that causes renal failure. (23) (55)

Lastly, some case reports have demonstrated the link between the use of synthetic hallucinogens and excited delirium. It is a rather uncommon complication of synthetic hallucinogens but very serious when it occurs. Excited delirium is recognized by severe agitation, aggression and violence, hyperreflexia, unexpected strength, and hyperthermia even leading to sudden death caused by a cardiopulmonary arrest. (37) (57)

1.4.2.1 Phenylalkylamines

The phenylalkylamines are the largest group of hallucinogens. Compounds within this class can be recognized by the typical phenyl ring with an ethylamine substituent on position 2 of the phenyl ring. (Figure 1.6) (37) The best-known substances within this class are without doubt amphetamine, methylenedioxy-methylamphetamine (MDMA), methamphetamine and naturally occurring mescaline. Some are probably better known by their “street name”, e.g., *Europa* or 2C-E. (58)

The phenylalkylamines can be further categorized into 3 different classes: the phenylisopropylamines, the phenethylamines, and the N-benzylphenethylamines or NBOMe. The phenylisopropylamines are the class of the amphetamines and DOx substances. The phenylethylamines contain the 2C-X compounds and mescaline as important members. (47) (59) The N-benzylphenethylamines made their way on the (illicit) market more recently. (49) The general structure of the compounds is shown in Figure 1.5.

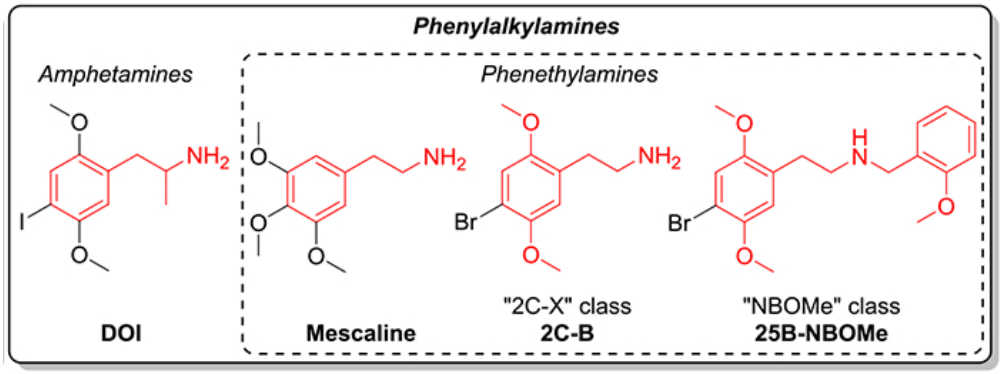

Figure 1.6: Structure of the phenylalkylamines where a subdivision can be made between the phenylisopropylamines containing DOx and amphetamines, the phenethylamines and the N-benzylphenetylamines. The general structure within each group is marked in red. Image used from (47).

1.4.2.2 Lysergamides

The lysergamides are a part of the indoleamine family (Figure 1.7) of hallucinogens and are derivatives of naturally occurring ergot alkaloids. The most famous compound within this class is LSD. The abbreviation of LSD comes from the German *Lyserg-Säure-Diäthylamid*. (47) Most lysergamides can be prepared easily with a few synthesis steps starting from naturally occurring ergot alkaloids. (60)

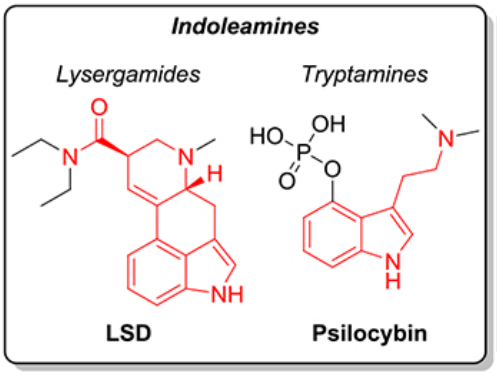

Figure 1.7: Structure of the indoleamines where a subdivision can be made between the lysergamides such as LSD and tryptamines as psilocybin. The general structure within each group is marked in red. Image used from (47).

1.4.2.3 Tryptamines

The tryptamines are mono-alkaloids and are also part of the indoleamines (Figure 1.7). They can be synthesized starting from the amino acid tryptophan. The general structure can be described as an indole ring (i.e., a benzene ring and a pyrrole ring) with an ethylamine side chain. (37) A few neurotransmitters, such as 5-HT (Figure 1.8) and melatonin are tryptamines but most of the naturally occurring tryptamines are hallucinogens (e.g., psilocybin, DMT). (61) (62)

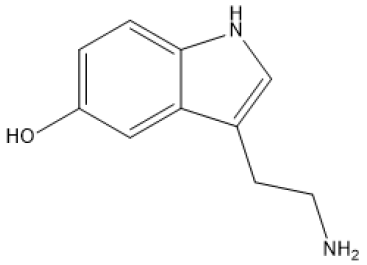

Figure 1.8: Structure of serotonin (5-HT) (This figure was created by the author using ChemDraw® Professional)

For the last 10 years a shift in recreational drug use can be noticed from well-known illicit drugs (e.g., cocaine, LSD, amphetamine, etc.) to the NPS. (62) These NPS have similar effects but with the advantage that they often exist in a legal grey space, may be cheaper and are often mislabelled so that users don't know what they are taking. (62) (63) As mentioned before, these NPS are usually either analogues of existing, controlled pharmaceuticals or newly synthesized substances. In this way these psychoactive substances can circumvent drug control legislation (e.g., in Canada). Since legislation in Belgium is done in substances groups, this country is better protected against the

introduction of new substances within each chemical group. Over the last few years, the demand for synthetic tryptamines has won popularity. Since there is also a lack of information on the pharmacology of these compounds, new insights on these newly developed synthetic tryptamines are urgently needed. (62)

In this dissertation the focus will be on position 4 and position 5 substituted tryptamines, specifically 4-AcO DMT, 4-AcO MPT, 5-MeO MiPT, 5-MeO AMT and 5-chlorotryptamine. Their activity will be compared using LSD as reference agonist. As stated by Pottie et al. (2020), a comparison of results regarding the potency and efficacy of the drugs, obtained using different experimental setups and methods is rather difficult and complicated. Therefore, when results are compared, they are ideally obtained using the same experimental paradigm. (64) Since the method that is used here (i.e., AequoScreen assay) is not as extensively used as some other methods, specific values for EC_{50} and E_{max} cannot be directly compared. However, because the assay used in the present study is based on measuring a chemiluminescent signal caused by Ca^{2+} flux, other assays that measure Ca^{2+} flux are the best measure to compare with the obtained EC_{50} and E_{max} values. (64) (65)

The assay that is widely used to determine Ca^{2+} flux is a fluorescence intensity plate reader (FLIPR) assay. In this assay, cells are loaded together with Ca^{2+} sensitive fluorescence dye in the wells of black multi-well plates with a clear bottom. Depending on which fluorescent dye is used, a different interpretation of the results is needed. Some dyes (i.e., fura-2) will cause a shift in their excitation spectrum to a different wavelength when Ca^{2+} binds, other dyes (i.e., fluo-3 and fluo-4) will cause an increase in fluorescence intensity at the same wavelength when Ca^{2+} binds. Some advantages to this method are that it is straightforward and can be automated easily. On the other side, this method also has some disadvantages. Fluorescence interference of other compounds can occur when performing the assay. Next to that, the assay is super sensitive to temperature changes. Lastly the dye needs to penetrate and be trapped in the cells to obtain good results. This can cause some problems when efflux of the dye happens. Because the temperature tends to fluctuate in the lab and to avoid potential fluorescence interference from the compounds themselves, the AequoScreen assay is somewhat easier to perform and the photoprotein is not able to diffuse out of the cells. This assay was chosen over the FLIPR assay. (19)

4-acetoxy-N,N-dimethyltryptamine (4-AcO DMT) or psilacetin (66) is a prodrug of psilocin just like psilocybin. It can be converted to psilocin by deacetylation. The advantage of 4-AcO DMT is that it can be easily synthesized and is more stable. (67) Since 4-AcO DMT is a prodrug, the pharmacology and toxicology of the compound *in vivo* are considered the same as psilocin. (68) But next to that psilacetin also has activity on various 5-HTRs itself. (66) 4-acetoxy-N-methyl-N-propyltryptamine (4-AcO MPT) is the second 4-substituted tryptamine that is going to be described. (69)

When looking at the pharmacology of both compounds, 4-AcO DMT and 4-AcO MPT seem to be potent drugs on the 5-HT_{2A}R and 5-HT_{2B}R with an EC50 value in the nanomolar range. When comparing the drugs, 4-AcO MPT is the more potent one. (67) Next to that, 4-AcO DMT is efficacious on the 5-HT_{2A}R, whereas other 4-acetylated tryptamines as 4-AcO MPT are highly efficacious on that receptor. When looking at the 5-HT_{2B}R, the efficacy of the 4-AcO DMT is very low, for 4-AcO MPT the efficacy is slightly higher. Both compounds are able to activate the 5-HT_{2A}R and the 5-HT_{2B}R and can thus be classified as psychedelics. (67) 4-AcO DMT has agonistic properties on the 5-HT_{2A}R but is classified as a partial agonist on the 5-HT_{2B}R. 4-AcO MPT can be classified as a full agonist on the 5-HT_{2A}R and as a partial agonist on the 5-HT_{2B}R.

5-methoxy-N-methyl-N-isopropyltryptamine (5-MeO MiPT) or better known under the name “Moxy” (70) is a 5-substituted tryptamine derivative that is closely related to 5-MeO DiPT or “foxy methoxy”. It is known that the compound binds to several 5-HTRs including 5-HT_{2A}R and 5-HT_{2B}R. (71) When looking at the pharmacology of this compound, 5-MeO MiPT seems to be a potent drug on the 5-HT_{2A}R with an EC_{50} value in the nanomolar range, but not on the 5-HT_{2B}R with an EC_{50} value in the micromolar range. The efficacy of 5-MeO MiPT on the 5-HT_{2A}R is high, but on the contrary the drug has a very low efficacy on the 5-HT_{2B}R. (70) (71) 5-MeO MiPT can be classified as a full agonist on the 5-HT_{2A}R, but as a weak partial agonist or even antagonist on the 5-HT_{2B}R since minimal effect can be seen when the receptor is activated.

5-methoxy-α-methyltryptamine (5-MeO AMT) or “Alpha-O” is a synthetic tryptamine that is similar to N,N-dimethyltryptamine (DMT) which is a naturally occurring tryptamine.

Over the last years a trend can be seen where 5-MeO AMT is occasionally sold under the name of LSD. (72) It is known that the compound is able to bind to several 5-HTR including 5-HT_{2A}R and 5-HT_{2B}R but little is known about the pharmacology of this tryptamine derivative. (71) (72) 5-MeO AMT seems to be a very potent drug on the 5-HT_{2A}R and the 5-HT_{2B}R with an EC_{50} value in the low nanomolar range. The efficacy of 5-MeO AMT is on the lower side on the 5-HT_{2A}R (73), but on the contrary the drug is highly efficacious on the 5-HT_{2B}R. (71) 5-MeO AMT can be classified as a partial agonist on the 5-HT_{2A}R but as a full agonist on the 5-HT_{2B}R.

5-chloro tryptamine is a relatively new tryptamine derivative. No research on the potency and efficacy of this compound has been done this far. Better insight into the activity of 5-chlorotryptamine is urgently needed.

A general overview of the E_{max} and EC_{50} values for the compounds on the 5-HT_{2A}R and 5-HT_{2B}R reported in the literature are given in Table 1.1 and Table 1.2.

Table 1.1: Reported E_{max} and EC_{50} values of different compounds on the 5-HT_{2A}R in literature for a corresponding assay.

5-HT_{2A}R	**Efficacy (E_{max}) (% 5-HT)**	**Potency (EC_{50})**	**Assay**
LSD (71) **(± SD)**	28% ± 10%	261 nM ± 150 nM	G_q mediated Ca^{2+} flux assay: FLIPR
5-HT (66) (67)	100%	2.4 nM 0.26 nM	G_q mediated Ca^{2+} flux assay: FLIPR
4-AcO DMT (± SEM) (67)	79.2% ± 0.7%	103 nM	G_q mediated Ca^{2+} flux assay: FLIPR
4-AcO MPT (± SEM) (67)	96.0% ± 1.1%	42.4 nM	G_q mediated Ca^{2+} flux assay: FLIPR

Table 1.1: Reported E_{max} and EC_{50} values of different compounds on the 5-HT_{2A}R in literature for a corresponding assay.

5-HT_{2A}R	Efficacy (E_{max}) (% 5-HT)	Potency (EC_{50})	Assay
5-MeO MiPT (± SD) (71)	83% ± 7%	23 nM ± 4 nM	G_q mediated Ca^{2+} flux assay: FLIPR
5-MeO AMT (± SD) (71)	60% ± 5%	2 nM ± 0.2 nM	G_q mediated Ca^{2+} flux assay: FLIPR
5-chloro tryptamine	Unknown	Unknown	No literature available

Table 1.2: Reported E_{max} and EC_{50} values of different compounds on the 5-HT_{2B}R in literature for a corresponding assay.

5-HT_{2B}R	Efficacy (E_{max}) (% 5-HT)	Potency (EC_{50})	Assay
LSD (15)	83%	40 nM	G_q mediated Ca^{2+} flux assay: FLIPR
5-HT (15) (66)	100%	1.4 nM 0.9 nM	G_q mediated Ca^{2+} flux assay: FLIPR
4-AcO DMT (± SEM) (67)	22.1% ± 1.0%	100 nM	G_q mediated Ca^{2+} flux assay: FLIPR
4-AcO MPT (± SEM) (67)	62.7% ± 1.2%	28.8 nM	G_q mediated Ca^{2+} flux assay: FLIPR
5-MeO MiPT (± SD) (71)	12% ± 7%	1.5 µM ± 0.9 µM	G_q mediated Ca^{2+} flux assay: FLIPR
5-MeO AMT (± SD) (71)	104 % ± 19 %	4 nM ± 1 nM	G_q mediated Ca^{2+} flux assay: FLIPR
5-chloro tryptamine	Unknown	Unknown	No literature available

1.5 STRUCTURE ACTIVITY RELATIONSHIP OF TRYPTAMINE DERIVATIVES ON THE 5-HT RECEPTOR

The structure activity relationship (SAR) of a compound on a certain receptor can be defined as the relationship between the chemical structure of that compound and the biological activity of the compound on a certain receptor, in this case the 5-HT_{2A}R and 5-HT_{2B}R. In this paragraph the SAR of 4-substituted and 5-substituted tryptamines will be described. In Figure 1.9 an overview of all tested tryptamine derivatives is given.

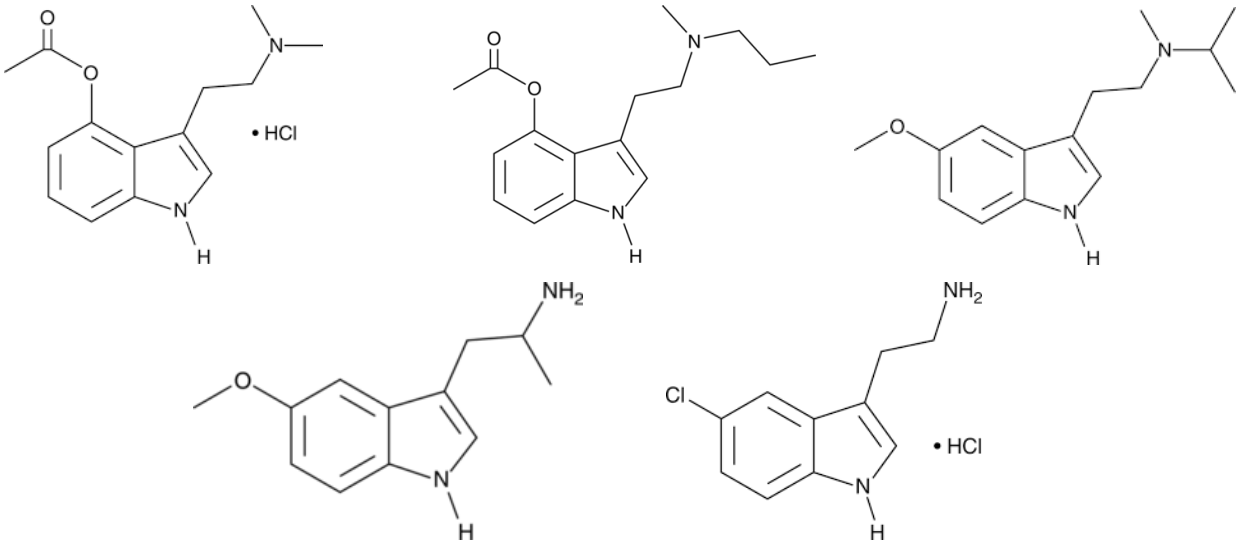

Figure 1.9: Chemical structure of the tested tryptamine derivatives (from left to right): 4-AcO DMT, image used from (74); 4-AcO MPT, image used from (69); 5-MeO MiPT, image used from (75); 5-MeO AMT, image used from (76) and 5-chloro tryptamine, image used from (77).

As a part of the SAR assessment, an evaluation of the influence of ring-substituent identity and position is needed. The phenyl ring can be substituted on several different positions. Substitution with a hydroxy, methoxy or acetoxy group on position 6 or 7 as compared to positions 4 or 5 causes a decrease in affinity of the compound's affinity for the receptor. Therefore, substitutions at positions 4 and 5 are better tolerated. (78)

This dissertation describes 4-substituted tryptamines and 5-substituted tryptamines. Both of the 4-substituted compounds contain an acetoxy group on position 4 in their chemical structure. *In vitro* data (i.e., receptor study) shows a lower potency of the acetylated compounds compared to the non-acetylated, hydroxylated compounds. (67) However, the acetylated compounds exhibit higher potency compared to the phosphorylated compounds. (66) Further investigation *in vivo* showed no significant differences between acetylated and non-acetylated compounds. (67) Additionally,

substitution on position 4 improves the compound's affinity compared to the non-substituted analogue. (71) Two out of the 5 tested tryptamines are substituted with a methoxy group on position 5. Methoxylation appears to increase affinity and potency in comparison with their non-methoxylated compounds on position 5 on the 5-HT_{2A}R. (71)

The next part of the structure that could influence the compound's activity is the ethylamine side chain. An α-methylation of the side chain, as seen in 5-MeO AMT, has little effect on the compound's affinity for the receptor. (78) The α -methylation itself seems to have little influence on the affinity but a big difference in affinity (twice as high) occurs for one optical isomer over the other. (78) (79) (80)

Lastly, the influence of alkylation of the terminal amine needs to be evaluated. Secondary and tertiary amines appear to have a high affinity for the 5-HT_{2A}R and 5-HT_{2B}R *in vitro*. In contrast, quaternary ammoniums have a lower affinity for the receptors *in vitro*. When evaluating these findings *in vivo*, only tertiary amines display high activity on the 5-HT_{2A}R. (66) The length of the alkyl substituents on N seems to have little effect on the potency of the compounds tested on 5-HT_{2A}R and 5-HT_{2B}R *in vitro*. However, when assessing the influence on the potency *in vivo*, bigger and bulkier alkyl chains on N appear to decrease the potency of the compounds. (67) Additionally, compounds with asymmetrical substitutions on N seem to have a greater activity compared to symmetrical substitutions on N. (71)

Since no studies have been conducted with 5-chlorotryptamine, the influence of the Cl substituent on position 5 on the affinity, efficacy or potency of the compound cannot be predicted.

2 OBJECTIVES

This thesis is conducted in cooperation with Health Canada, the federal department in Canada responsible for the maintenance and improvement of the health of the Canadian population. Health Canada oversees all health-related matters within the country, including funding various research projects, establishing guidelines and health-related legislation to be followed in Canada, and granting marketing authorisation for new medicinal products, etc. (81) During this research, the tryptamine derivatives that were tested were selected based on drug monitoring programs in cooperation with Health Canada.

Health Canada aims to gain insight into the activity of these compounds. Since the tested substances are tryptamine derivatives, they are classified as hallucinogens. As mentioned before in paragraph 1.4.2, the primary mediator of hallucinogenic effects is the interaction of these compounds with the 5-HT_{2A}R. Additionally, the contribution of the 5-HT_{2B}R cannot be overlooked. Getting insight into the activity on the 5-HT_{2B}R is also crucial for estimating the potential harm these compounds may cause to the heart, as discussed in paragraphs 1.3.2 and 1.4.2. Therefore, the primary objective of this thesis is to gain insight in the activity of hallucinogens, specifically tryptamine derivatives on the 5-HT_{2A}R and the 5-HT_{2B}R. Due to time constraints, other important mediators of hallucinogenic effects (i.e., 5-HT_{1A}R and 5-HT_{2C}R) could not be investigated in this project.

After determining the activity of these compounds, an assessment of their potential dangers can be made. Since these compounds are circulating on the illicit market, it is crucial for Health Canada to be aware of their activity. Overstimulation of the 5-HT_{2A}R and 5-HT_{2B}R or an overdose could have disastrous consequences for the users. Therefore, the second objective of this study is to gain insight into the dangers of these substances when they are freely available on the (illicit) market.

Based on all the information collected during this research and additional information that will be gathered in future projects, Health Canada will decide whether these substances should be scheduled in the Controlled Drugs and Substances Act. As mentioned in paragraph 1.4.1 Canada follows a scheduling system in which each drug is separately listed in a schedule (I to IX). On the other hand, Belgium does not follow a scheduling system but describes substance groups (including the tryptamines) instead.

The tryptamine derivatives studied in this research are already included in the Belgian legislation, but they have not been scheduled in Canada yet. If the activity of these tryptamine derivatives on the 5-HT_{2A}R and the 5-HT_{2B}R indicates that these compounds could be potentially dangerous, they should be scheduled as quickly as possible. Therefore, the third objective of this thesis is to determine whether Health Canada should eventually schedule these tryptamine derivatives in one of the schedules.

3 MATERIALS AND METHODS

3.1 CELL CULTURE

All reagents were purchased from ThermoFisher Scientific (Mississauga, ON, Canada), unless specifically noted. During these experiments AequoScreen® CHO-K1 cells stably expressing either human 5-HT_{2A}R (h5-HT_{2A}R) or human 5-HT_{2B}R (h5-HT_{2B}R) were used. They were purchased from Perkin-Elmer (Woodbridge, ON, Canada). The cells were cultivated in Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12 (DMEM/F-12 medium), a 1:1 mixture of DMEM and Ham's F-12 consisting of high concentrations of glucose, various amino acids and vitamins (product number: 11330-032) supplemented with 10% foetal bovine serum (FBS) and penicillin-streptomycin antibiotic solution (1%) to prevent bacterial contamination of the medium. (82)

A new CHO-K1 h5-HT_{2A}R or h5-HT_{2B}R cell culture was set up using already existing cell cultures from a fellow student by passaging the cells using a 2.5% trypsin solution into new 25 mL flasks. The original cell medium was removed from the flasks. Then the cells were washed using phosphate buffer saline (PBS) to remove the remaining medium on the cells. After washing of the cells, PBS was removed from the flask. Next 1 mL trypsin (2.5%) was added, and the flasks were incubated at 37°C and 5% CO_2 for 7 minutes. After 7 minutes, the flasks were swirled and were again incubated again at 37°C and 5% CO_2 for 7 minutes. Meanwhile, new flasks were prepared by adding 3 mL of fresh DMEM/F-12 medium containing 10% FBS and 1% penicillin-streptomycin to each flask.

After the incubation, 9 mL of fresh medium was added to the old flasks to inactivate trypsin and collect the cells. The content of each flask was transferred into a falcon tube and centrifuged for 10 min, 369 x g and at 21°C. After centrifugation, the medium was removed, and the cell pellet was resuspended in fresh medium. One mL of medium containing cells was transferred into the new flask. The new flasks reached a final volume of 4 mL and were incubated at 37°C and 5% CO_2. A new passage was performed when the cells reached confluency.

3.2 AEQUOSCREEN ASSAY

3.2.1 Principal of the AequoScreen Assay

Using the AequoScreen Assay or Aequorin Calcium release assay, the activity of psychedelics on the 5-HT_{2A}R or 5-HT_{2B}R can be quantified. The CHO-K1 cells used are equipped with an apo-enzyme called apoaequorin. Activation of this enzyme requires the hydrophobic prosthetic group coelenterazine h. After binding, the active enzyme aequorin is formed and capable for binding 3 Ca^{2+} ions. Upon Ca^{2+} binding, aequorin oxidizes coelenterazine into coelenteramide, along with the production of CO_2 and eventually the emission of light when excited coelenteramide relaxes to its lowest energy state (Figure 2.1). The consumption of aequorin is proportional to the Ca^{2+} concentration in the cell. Since the light emission results from the activation of the apoaequorin enzyme, it is also proportional to the Ca^{2+} concentration. Therefore, this assay is a reliable tool to measure the intracellular Ca^{2+} flux. The emitted light can be detected using a luminometer. (65) (83) (84)

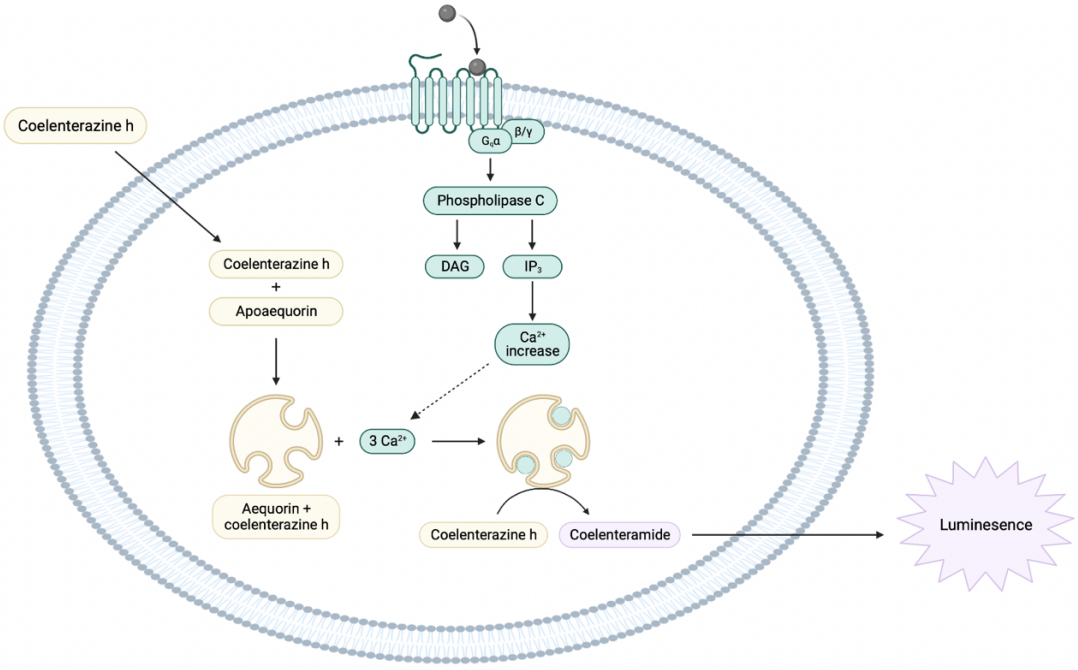

Figure 3.2: AequoScreen Assay principle. This figure was created by the author using BioRender.com.

As mentioned in paragraph 1.2.1 and 1.3 activation of the 5-HT_{2A}R and 5-HT_{2B}R causes recruitment of the heterotrimeric G-protein to the intracellular surface of the GPCR. Eventually, the Gα monomer activates the effector protein PLCβ. PLCβ hydrolyses PIP_2 to the second messengers IP_3 and DAG. IP_3 opens Ca^{2+} channels in the endoplasmatic

reticulum of the cell and causes release of Ca^{2+} in the cytosol. The Ca^{2+} flux in the cytosol caused by activation of the receptor by psychedelics can thus be determined using the AequoScreen Assay.

3.2.2 The AequoScreen assay

All drugs were purchased from Cayman Chemical (Ann Arbor, MI, USA) and all reagents were purchased from ThermoFisher Scientific (Mississauga, ON, Canada), unless specifically noted. The AequoScreen assay was an experiment performed over 2 days. On day 1 cells were passaged into a 96-well plate. After an overnight incubation the assay was performed on day 2.

The experiment is started when a sufficient number of cells were grown. During every experiment an equivalent of 4 flasks of 25 mL with confluent cells were used. A new passage of those cells had to be performed. As explained in paragraph 3.1 the old medium was removed from the flasks, the cells were washed with PBS and 1 mL of a 2,5% trypsin solution was added to each flask. After 2 times 7-minute incubation, 5 mL of fresh DMEM/F-12 medium was added to the first flask. The walls of the flask were washed thoroughly to make sure all the cells were suspended in the medium. The medium was taken out of the first flask and transferred into the next flask. The same procedure was repeated until the medium was transferred through the 4 flasks. The obtained medium had a high number of cells.

The medium was transferred into a falcon tube and was centrifuged for 10 min, 369 x g and at 21°C. After centrifugation, the medium was removed, and the cell pellet was resuspended into 3 mL of fresh DMEM/F-12 medium. Ten µL of the resuspended cell solution was transferred on a plate (counting slides, dual chamber for cell counter, BIO RAD). Using an automatic cell counter (TC20™ Automated Cell Counter, BIO RAD), the number of cells (i.e., cells/ mL) were counted.

The cells were transferred to a 96-well white-walled, white-bottomed tissue culture microplate. Each well contained 2 x 10^4 cells and had a final volume of 100 µL. By using

formula (3.1), there could be calculated how much of the resuspended solution needed to be transferred into the each well of the 96-well microplate.

$$V = \frac{1\ mL\ x\ 2\ x\ 10^4 cells}{\#cells} \quad (3.1)$$

Where: V = volume of resuspended solution (mL)

$\#cells$ = number of cells in the resuspended solution (cells)

Fresh DMEM/F-12 medium was added to each well to reach a final volume of 100 µL per well. One full 96-well plate and 9 rows of a second 96-well plate were needed to be able to test all drugs the next day. The plates were incubated overnight at 37°C and 5% CO_2.

After the overnight incubation, the DMEM/F-12 medium was removed from each well and replaced with 50 µL of Opti-MEM medium with 1% FBS, containing 5 µM coelenterazine h (Promega Corporation, Madison, WI, USA). The cells were incubated at room temperature, outside of the incubator for 4 hours. Since coelenterazine h is a light sensitive molecule, the 96-well microplates were protected from light using aluminium foil. (85) During this experiment, coelenterazine h is used instead of coelenterazine. The structure of coelenterazine h is slightly different where one hydroxyl group form coelenterazine is replaced by a hydrogen. This causes a 10-fold increase in luminescence intensity in comparison with coelenterazine. Therefore, coelenterazine h is preferred over coelenterazine. (85) (86)

During the 4-hour incubation at room temperature, a drug plate was made according to the 96-well plates in Figure 2.2 and Figure 2.3. During one experiment, 5 tryptamine derivatives i.e., 4-AcO DMT, 4-AcO MPT, 5-MeO AMT, 5-MeO MiPT and 5-chlorotryptamine were tested next to LSD (Supelco, Mississauga, ON, Canada), which functioned as reference agonist and 5-HT (Sigma-Aldrich, Oakville, ON, Canada), which was used as an internal check to ensure that the obtained data was comparable to already published data. As an additional positive control, 4 wells contained 200 µM digitonin (Sigma-Aldrich, Oakville, ON, Canada). Digitonin is a non-ionic detergent that is able to solubilize certain membrane proteins as cholesterol and enhance permeabilization of the plasma membrane. (87) (88) This substances was used as a measure of the maximal

assay response. (89) Three wells were filled with vehicle (i.e., 10% DMSO in PBS) and 3 wells were left empty, they functioned as a blank. Each well had a final volume of 50 µL.

For each tryptamine derivative and LSD, a 20 µM stock solution in 10% DMSO in PBS was made. 50 µL of the 20 µM stock solution was transferred into each well of row C. 10-fold dilutions were made starting from row C working downwards (as shown Figure 1.2 and 2.2). This was done by adding 45 µL of 10% DMSO in PBS to each remaining well in row D to H. 5 µL of the solution in row C was transferred into the wells in row D. Following that, again 5 µL of the solution in row D was transferred into the wells in row E. This process was repeated until the desired amount of dilutions were reached. A concentration range from 20 µM to 0.2 nM was obtained in row C to H.

For 5-HT, a 2 mM stock solution in 10% DMSO in PBS was made. 0.5 µL of the 2 mM stock solution was transferred into each well of row C and 49.5 µL of 10% DMSO in PBS was added to reach a final volume of 50 µL in each well. Then again for 5-HT, a 10-fold dilution was made starting from row C working downwards. A concentration range from 20 µM to 0.2 nM was obtained in row C to H.

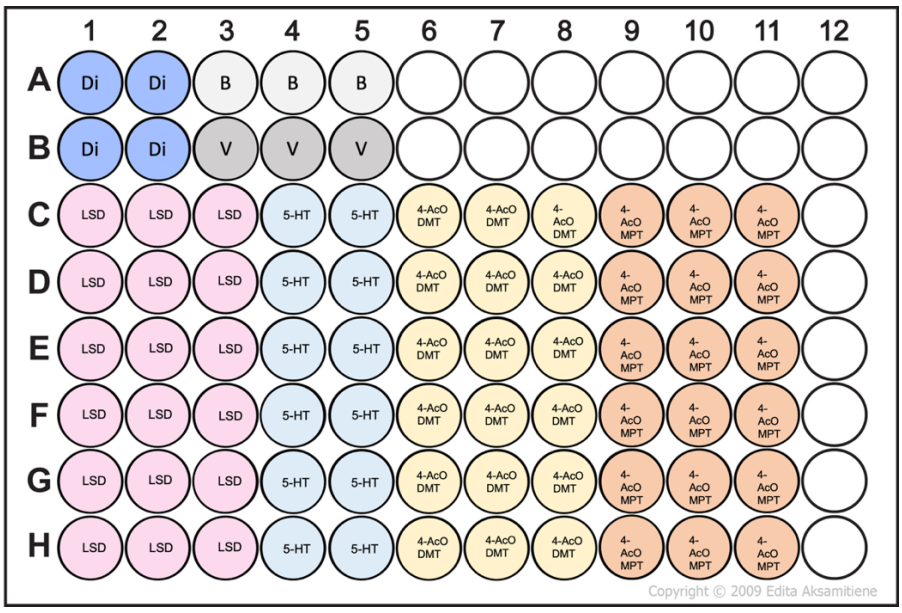

Figure 2.2: Drug plate A: digitonin (control), blank (empty wells), vehicle, LSD (reference agonist), 5-HT (control), 4-AcO DMT and 4-AcO MPT. The compounds are run in duplicate or triplicate with a concentration range from 0.2 nM – 20 µM and a final volume of 50 µL. This figure was created by the author using Microsoft PowerPoint. (Askamitiene, 2009)

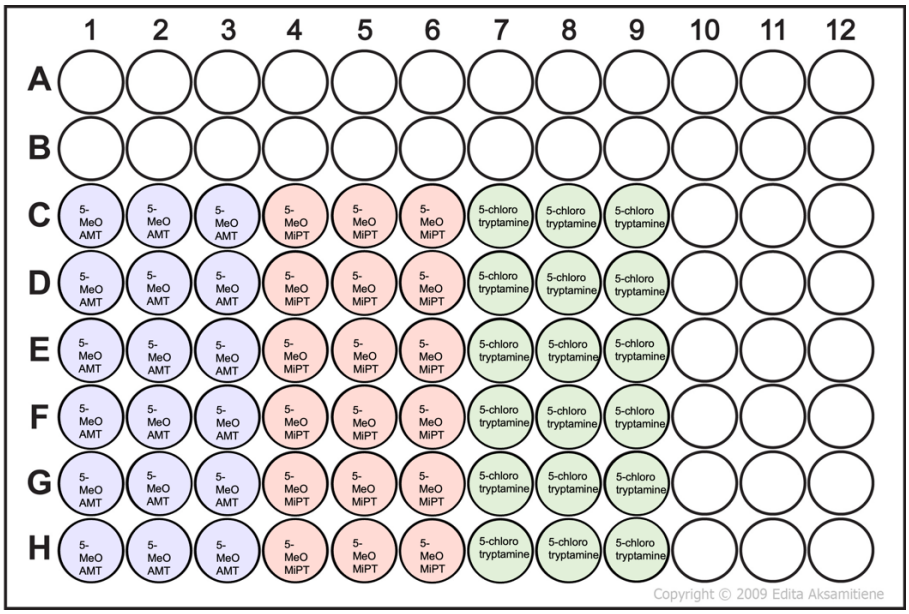

Figure 2.3: Drug plate B: 5-MeO AMT, 5-MeO MiPT and 5-chlorotryptamine. The compounds are run in triplicate with a concentration range from 0.2 nM – 20 µM and a final volume of 50 µL. This figure was created by the author using Microsoft PowerPoint. (Aksamitiene, 2009)

After the 4-hour incubation, the content (i.e., 50 µL) of the 96-well drug plates was transferred into the 96-well white-walled, white-bottomed tissue culture microplate containing the cells in 50 µL of Opti-MEM medium with 1% FBS and 5 µM coelenterazine h. The final volume in each well was 100 µL. By adding 50 µL of drug solution to each well the final concentration in the wells for the tryptamine derivatives, LSD and 5-HT in the wells ranged from 10 µM to 0.1 nM. The final digitonin concentration was 100 µM.

After transferring the content of the drug plate, the 96-well white-walled, white-bottomed tissue culture microplate was centrifuged for 1 min at 369 x g, 21°C. Immediately after centrifugation the chemiluminescent signal was read with an integration time of 2000 ms using a standard luminescence plate reader (Synergy™ HT Cytation microplate reader, BioTek*)* operating with Gen5 software (BioTek).

3.2.3 Statistical analyses

The obtained data was analysed using Prsim 9, where a sigmoidal log(concertation)-response curve was constructed using the 3 parametric nonlinear regression model. The goal was to determine the potency and the efficacy of each compound by deriving the pEC_{50} value and E_{max} values, respectively.

The obtained data for the tryptamine derivatives was transformed as fold over vehicle. Each dataset was transformed as follows: Y = X/K with a different K for each dataset. For each tryptamine derivative, LSD, and 5-HT the value for K was set to the bottom value (i.e., baseline, vehicle) of the corresponding dataset. After transforming fold over vehicle, the data was normalized using the data obtained from LSD, the reference agonist. The bottom value of LSD was set to 0%, and the top value of LSD was set to 100%.

The mean of all obtained data points for each concentration was used to construct the final sigmoidal log(concertation)-response curve. For every data point, the error bars were constructed using the standard error of the mean (SEM). Outliers were excluded during analysis if they were greater than 2 SD away from the mean.

After constructing the three-parameter, nonlinear regression log(concentration)-response curves, the data was submitted to a one-way ANOVA statistical test followed by a post-hoc Bonferroni's test. This was done to determine if a statistically significant difference between the efficacy and potency of the tested tryptamine derivatives and LSD, the reference agonist, could be observed.

4 RESULTS

The activity of 5 tryptamine derivatives i.e., 4-AcO DMT, 4-AcO MPT, 5-MeO MiPT, 5-MeO AMT and 5-chloro tryptamine is being determined using the AequoScreen Assay. The experiment is performed on the 5-HT_{2A}R and the 5-HT_{2B}R. Optimally, 6 independent experiments on each compound are performed with each compound tested in triplicate during each experiment. For each compound a three-parameter non-linear regression sigmoidal log(concentration)-response curve is shown alongside the corresponding E_{max} values and pEC_{50} values as stated in paragraph 3.2.3.

4.1 ACTIVITY ON THE 5-HT_{2B} RECEPTOR

Firstly, the activity of LSD, 5-HT and the tryptamine derivatives was tested on the 5-HT_{2B}R. Six independent experiments were performed. Each compound was tested in triplicate in every experiment with the exemption of 5-HT which was tested in duplicate due to the amount of tryptamine derivatives that needed to be tested.

4.1.1 Activity of 5-HT and LSD on the 5-HT_{2B} receptor

In each experiment the efficacy (E_{max}) and potency (pEC_{50}) of 5-HT and LSD were determined. 5-HT was used as an internal check for the experiment ensure that the obtained data is comparable to already published data. If the obtained data was in line with literature, then the conclusions drawn from these experiments were trustworthy. LSD was used as a reference agonist.

After testing the activity of 5-HT and LSD on the 5-HT_{2B}R a three-parameter non-linear regression sigmoidal log(concentration)-response curve could be constructed as shown in Figure 4.1. The data was transformed fold over vehicle and normalized in function of % LSD. The E_{max} values and pEC_{50} values for 5-HT and LSD are given in Table 4.1.

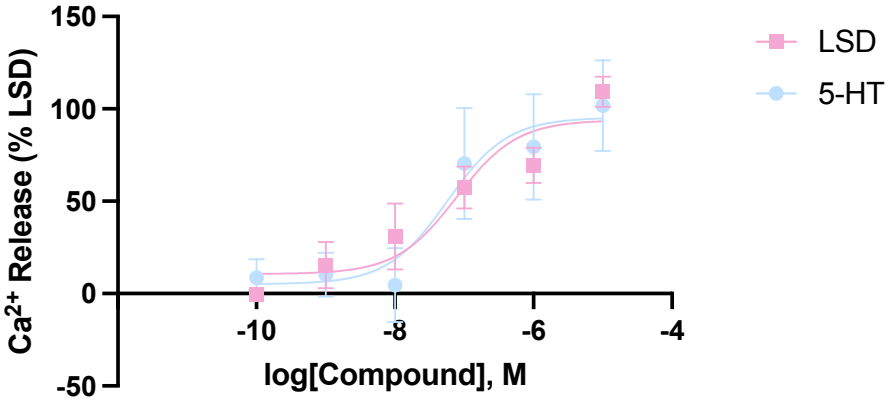

Figure 4.1: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 5-HT as internal check in blue. The reported values are mean ± SEM and n = 6 for LSD and 5-HT.

4.1.2 Activity of the 4-substituted tryptamine derivatives on the 5-HT_{2B} receptor

In each experiment the efficacy (E_{max}) and potency (pEC_{50}) of the 4-substituted tryptamine derivatives 4-AcO DMT and 4-AcO MPT were determined. Testing the activity of these compounds resulted in a three-parameter non-linear regression sigmoidal log(concentration)-response curves. The data was transformed fold over vehicle and normalized in function of % LSD. The sigmoidal log(concentration)-response curve of 4-AcO DMT in comparison to LSD is shown in Figure 4.2 and the sigmoidal log(concentration)-response curve of 4-AcO MPT in comparison to LSD is shown in Figure 4.3. The E_{max} values and pEC_{50} values of LSD, 4-AcO DMT and 4-AcO MPT are given in Table 4.1.

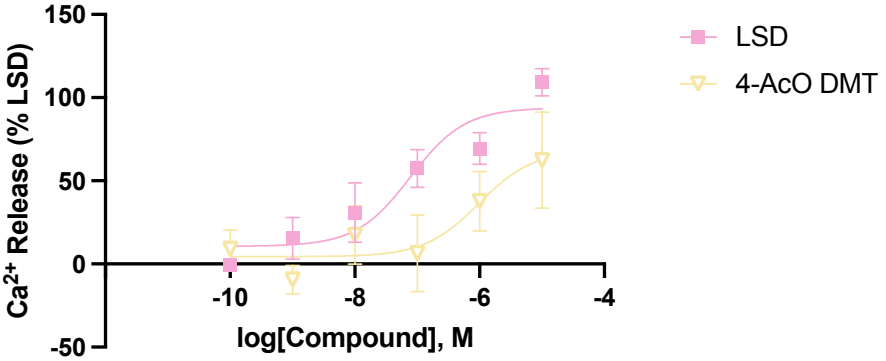

Figure 4.2: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 4-AcO DMT in yellow. The reported values are mean ± SEM and n = 6 for LSD and 4-AcO DMT.

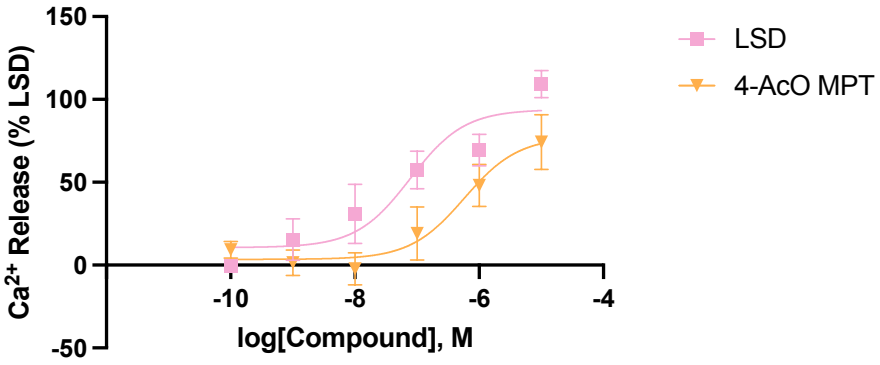

Figure 4.3: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 4-AcO MPT in orange. The reported values are mean ± SEM and n = 6 for LSD and 4-AcO MPT.

4.1.3 Activity of the 5-substituted tryptamine derivatives on the 5-HT_{2B} receptor

In each experiment the efficacy (E_{max}) and potency (pEC_{50}) of the 5-substituted tryptamine derivatives 5-MeO MiPT, 5-MeO AMT and 5-chloro tryptamine were determined. Testing the activity of those compounds resulted in a three-parameter non-linear regression sigmoidal log(concentration)-response curves. The data was transformed fold over vehicle and normalized in function of % LSD. The sigmoidal log(concentration)-response curve of 5-MeO MiPT in comparison to LSD is shown in Figure 4.4, the sigmoidal log(concentration)-response curve of 5-MeO AMT in comparison to LSD is shown in Figure 4.5 and the sigmoidal log(concentration)-response curve of 5-chloro tryptamine in comparison to LSD is shown in Figure 4.6. The E_{max} values and pEC_{50} values of LSD, 5-MeO MiPT, 5-MeO AMT, and 5-chloro tryptamine are given in Table 4.1.

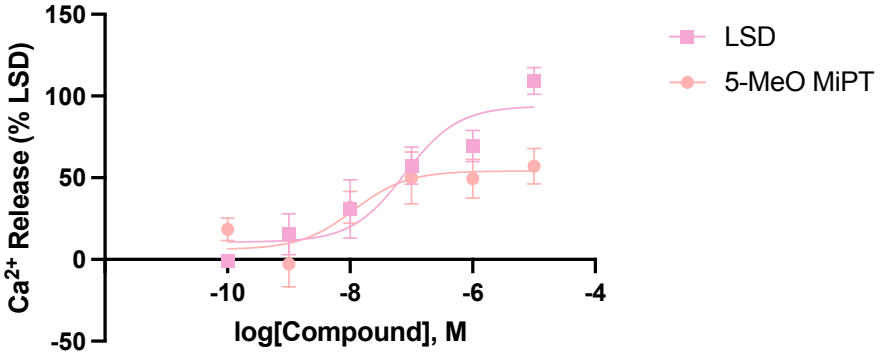

Figure 4.4: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 5-MeO MiPT in red. The reported values are mean ± SEM and n = 6 for LSD and n = 5 for 5-MeO MiPT.

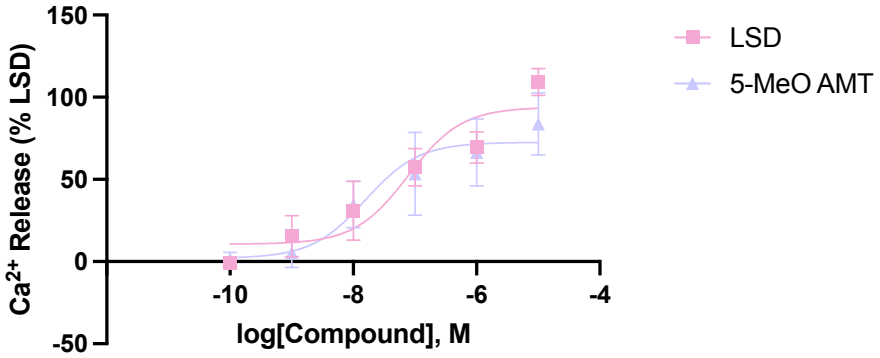

Figure 4.5: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 5-MeO AMT in purple. The reported values are mean ± SEM and n = 6 for LSD and 5-MeO AMT.

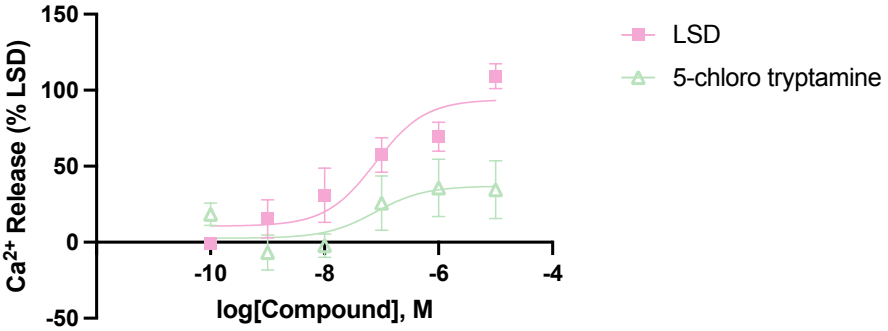

Figure 4.6: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 5-chloro tryptamine in green. The reported values are mean ± SEM and n = 6 for LSD and 5-chloro tryptamine.

Table 4.1: Reported E_{max} and pEC_{50} for LSD, 5-HT, and the tryptamine derivatives representing the efficacy and potency of the compounds on the $5\text{-}HT_{2B}R$.

	Efficacy (E_{max} ± SEM)	**Potency (pEC_{50} ± SEM [nM])**
LSD	93.91% ± 9.55%	7.109 ± 0.297 [77.85 nM]
5-HT	95.32% ± 16.6%	7.242 ± 0.508 [57.34 nM]
4-AcO DMT	68.30% ± 25.7%	6.013 ± 0.707 [969.6 nM]
4-AcO MPT	77.36% ± 14.2%	6.238 ± 0.328 [577.9 nM]
5-MeO MiPT	54.18% ± 7.71%	7.943 ± 0.521 [11.40 nM]
5-MeO AMT	72.75% ± 11.0%	7.808 ± 0.493 [15.58 nM]
5-chloro tryptamine	36.97% ± 11.7%	7.111 ± 0.880 [77.51 nM]

4.1.4 Activity of norpsilocin on the 5-HT_{2B} receptor

Next to the planned compounds to be tested, an additional compound, norpsilocin, was also tested. Norpsilocin is another tryptamine derivative that is recognized by the secondary amine group on the ethylamine sidechain and hydroxyl group on position 4 of the indole ring as seen in Figure 4.7. It is the active metabolite of baeocystin. Both compounds can be found in psilocybin-containing mushrooms or "magic mushrooms". (66)

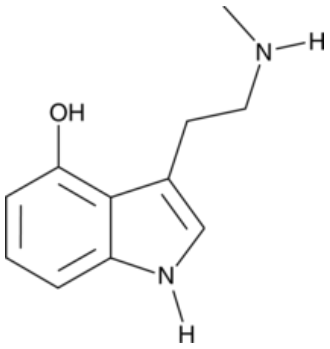

Figure 4.7: Chemical structure of norpsilocin (Cayman Chemical, 2023)

This compound was tested in one of the first experiments since 5-MeO MiPT was not available in the lab at that time due to supply issues. Norpsilocin was chosen as an alternative for this compound.

In this experiment the efficacy (E_{max}) and potency (pEC_{50}) of norpsilocin were determined. Testing the activity of this compound resulted in a three-parameter non-linear regression sigmoidal log(concentration)-response curve. The data was transformed fold over vehicle and normalized in function of % LSD. The sigmoidal log(concentration)-response curve of norpsilocin in comparison to LSD is shown in Figure 4.8. The E_{max} values and pEC_{50} values of LSD and norpsilocin are given in Table 4.2.

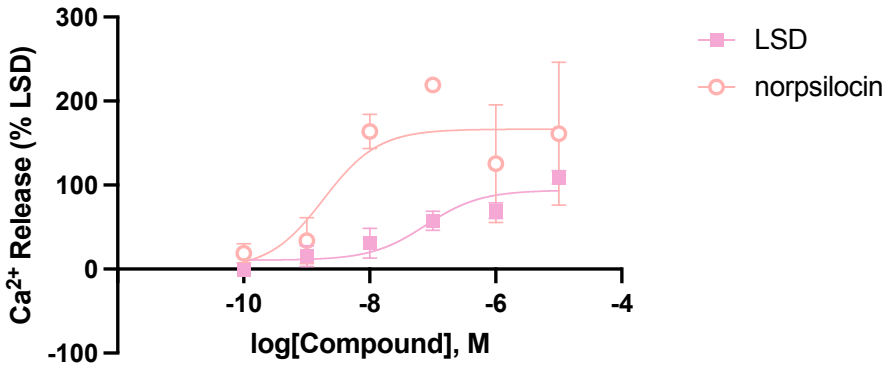

Figure 4.8: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log [Compound], M. LSD as the reference agonist in pink and norpsilocin in red. The reported values are mean ± SEM and n = 6 for LSD and n = 1 for norpsilocin.

Table 4.2: Reported E_{max} and EC_{50} for LSD and norpsilocin on the 5-HT_{2B}R representing the efficacy and potency of the compounds.

	Efficacy (E_{max} ± SEM)	**Potency (pEC_{50} ± SEM [nM])**
LSD	93.91% ± 9.55%	7.109 ± 0.297 [77.85 nM]
norpsilocin	166.5% ± 21.7%	8.731 ± 0.545 [1.857 nM]

4.2 ACTIVITY ON THE 5-HT_{2A} RECEPTOR

Secondly, the activity of LSD, 5-HT, and the tryptamine derivatives was tested on the 5-HT_{2A}R. Due to time constraints only 1 adequate, independent experiment could be performed instead of 6 independent experiments. Each compound was tested in triplicate with the exemption of 5-HT which was tested in duplicate due to the amount of tryptamine derivatives that needed to be tested.

4.2.1 Activity of LSD and 5-HT on the 5-HT_{2A} receptor

In each experiment the efficacy (E_{max}) and potency (pEC_{50}) of 5-HT and LSD were determined. As mentioned before in paragraph 3.2.2 and 4.1.1, 5-HT was used as an internal check and LSD was used as reference agonist.

After testing the activity of 5-HT and LSD on the 5-HT_{2A}R a three-parameter non-linear regression sigmoidal log(concentration)-response curve could be constructed as

shown in Figure 4.9. The data was transformed fold over vehicle and normalized in function of % LSD. The E_{max} values and pEC_{50} values of 5-HT and LSD the are given in Table 4.3.

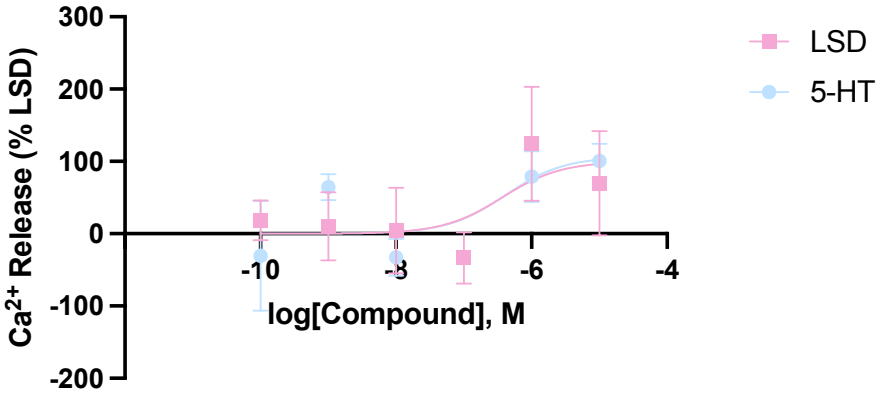

Figure 4.9: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 5-HT as internal check in blue. The reported values are mean ± SEM and n = 1 for LSD and 5-HT.

4.2.2 Activity of the 4-substituted tryptamine derivatives on the 5-HT_{2A} receptor

In each experiment, the efficacy (E_{max}) and potency (pEC_{50}) on the 5-HT_{2A}R of the 4-substituted tryptamine derivatives 4-AcO DMT and 4-AcO MPT were determined. Testing the activity of those compounds resulted in a three-parameter non-linear regression sigmoidal log(concentration)-response curves. The data was transformed fold over vehicle and normalized in function of % LSD. The sigmoidal log(concentration)-response curve of 4-AcO DMT in comparison to LSD is shown in Figure 4.10 and the sigmoidal log(concentration)-response curve of 4-AcO MPT in comparison to LSD is shown in Figure 4.11. The E_{max} values and pEC_{50} values of LSD, 4-AcO DMT and 4-AcO MPT are given in Table 4.3.

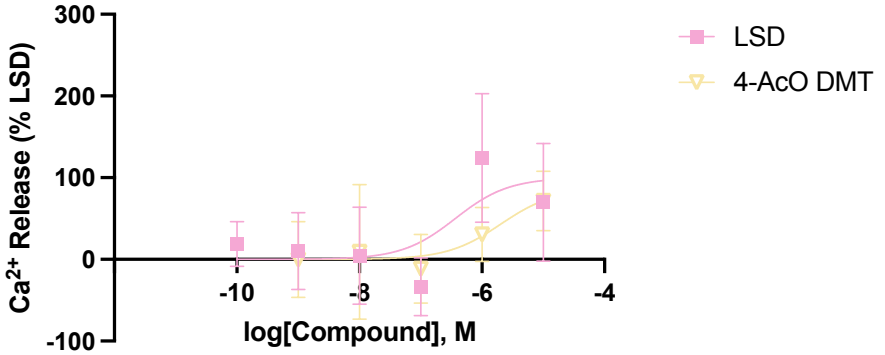

Figure 4.10: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 4-AcO DMT in yellow. The reported values are mean ± SEM and n = 1 for LSD and 4-AcO DMT.

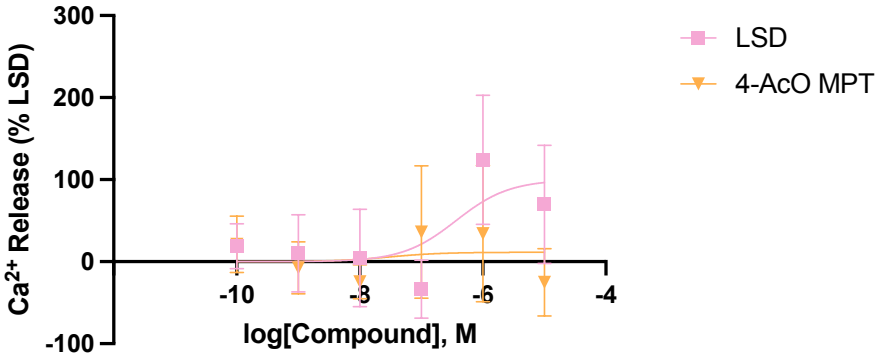

Figure 4.11: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 4-AcO MPT in orange. The reported values are mean ± SEM and n = 1 for LSD and 4-AcO MPT.

4.2.3 Activity of the 5-substituted tryptamine derivatives on the 5-HT_{2A} receptor

In each experiment the efficacy (E_{max}) and potency (pEC_{50}) of the 5-substituted tryptamine derivatives 5-MeO MiPT, 5-MeO AMT, and 5-chloro tryptamine were determined. Testing the activity of those compounds resulted in a three-parameter non-linear regression sigmoidal log(concentration)-response curves. The data was transformed fold over vehicle and normalized in function of % LSD. The sigmoidal log(concentration)-response curve of 5-MeO MiPT in comparison to LSD is shown in Figure 4.12, the sigmoidal log(concentration)-response curve of 5-MeO AMT in comparison to LSD is shown in Figure 4.13 and the sigmoidal log(concentration)-response curve of 5-chloro tryptamine in comparison to LSD is shown in Figure 4.14. The E_{max} values and pEC_{50} values of LSD, 5-MeO MiPT, 5-MeO AMT and 5-chloro tryptamine are given in Table 4.3.

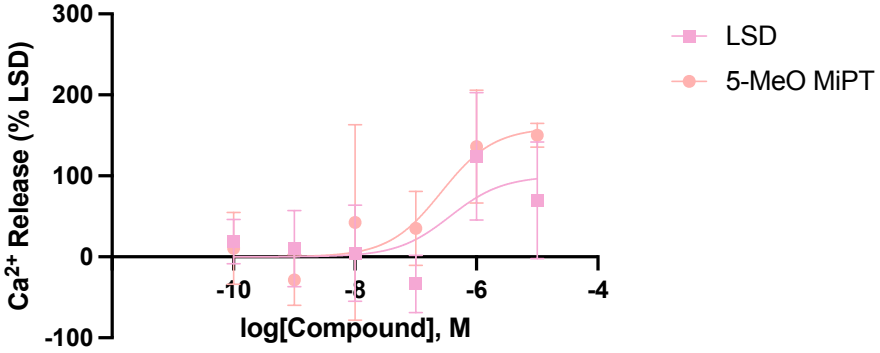

Figure 4.12: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 5-MeO MiPT in red. The reported values are mean ± SEM and n = 1 for LSD and 5-MeO MiPT.

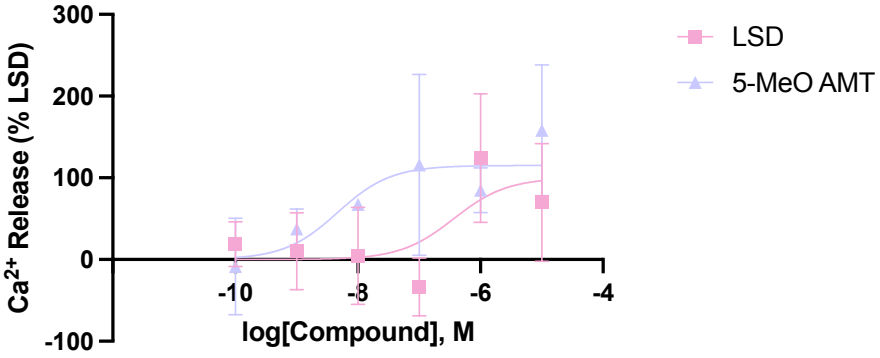

Figure 4.13: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 5-MeO AMT in purple. The reported values are mean ± SEM and n = 1 for LSD and 5-MeO AMT.

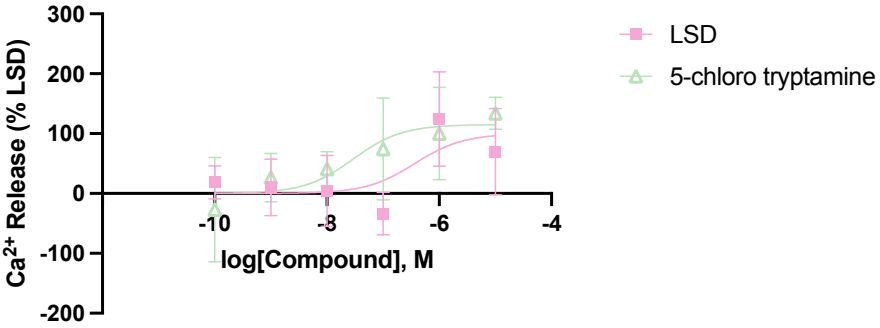

Figure 4.14: Three-parameter sigmoidal log(concentration)-response curve: Ca^{2+} release (% LSD) in function of log[Compound], M. LSD as the reference agonist in pink and 5-chloro tryptamine in green. The reported values are mean ± SEM and n = 1 for LSD and 5-chloro tryptamine.

Table 4.3: Reported E_{max} and pEC_{50} for LSD, 5-HT, and the tryptamine derivatives representing the efficacy and potency of the compounds on the $5\text{-}HT_{2A}R$.

	Efficacy (E_{max} ± SEM)	**Potency (pEC_{50} ± SEM [nM])**
LSD	100% ± 39.6%	6.435 ± 0.878 [367.1 nM]
5-HT	106.8% ± 47.5%	6.384 ± 1.112 [413.4 nM]
4-AcO DMT	86.19% ± 53.2%	5.694 ± 0.981 [2021 nM]
4-AcO MPT	11.46% ± 23.5%	7.528 ± 6.331 [29.64 nM]
5-MeO MiPT	159.7% ± 38.1%	6.579 ± 0.497 [263.5 nM]
5-MeO AMT	115.2% ± 22.3%	8.331 ± 0.683 [4.665 nM]
5-chloro tryptamine	115.2% ± 25.3%	7.531± 0.677 [29.47 nM]

5 DISCUSSION

After determining the activity of LSD, 5-HT, and the tryptamine derivatives on the 5-HT_{2B}R the results were compared with the numbers reported in the literature for these compounds. As mentioned in paragraph 1.4.2.3, the activity was determined using the AequoScreen Assay which has a different experimental setup than the FLIPR assay.

Since the data was normalized to percentage LSD, the efficacy of LSD was set to approximately 100%. When looking at the potency of LSD determined by the EC_{50} value, LSD had a potency of 77.85 nM. McCorvy et al. (2018) reported a potency of 40 nM which is in the lower nanomolar range similar to the measured value for LSD. (15) The obtained results for LSD were consistent with literature.

A one-way ANOVA test, followed by a post-hoc Bonferroni's tested was performed on the data to determine if there was a statistically significant difference in efficacy and potency between LSD, the reference agonist, and the tryptamine derivatives. According to this statistical analysis, no statistically significant difference was observed in the efficacy of LSD compared to any of the tryptamine derivatives. Additionally, no statistically significant difference was found in the potency of LSD compared to any of the tryptamine derivatives. These findings indicate that all compounds were able to at least emulate the efficacy and potency of LSD. The analysis of the measured values was based on the mean values reported for E_{max} and EC_{50}.

During the assays, 5-HT was used as an internal check as previously mentioned in paragraph 4.1.1. The E_{max} value obtained after testing the activity on the 5-HT_{2B}R was 95.32%. When comparing it to the literature, it was found that 5-HT was a stronger full agonist on the 5-HT_{2B}R compared to LSD. These experiments indicated that 5-HT was more efficacious than LSD, and the obtained efficacy for this compound was aligned with the literature. The obtained EC_{50} value for 5-HT was 57.34 nM. McCorvy et al. (2018) reported a potency of 1.4 nM for 5-HT on the 5-HT_{2B}R which was in the lower nanomolar range, similar to the measured value. (15) The obtained potency for 5-HT was consistent with literature.

Five tryptamine derivatives were tested. Firstly, the analysis and the comparison of the data with literature for the 4 substituted tryptamines were conducted. 4-AcO DMT appeared to be a partial agonist on the 5-HT_{2B}R with an efficacy of 68.30%. Klein et al. (2021) reported an efficacy of 22.10% and classified 4-AcO DMT as a weak partial agonist on the 5-HT_{2B}R. (67) The obtained efficacy was considerably higher than the reported efficacy. Therefore, there cannot be said with certainty that the obtained efficacy was in line with the reported efficacy, but the compound was classified as a partial agonist in both cases. An explanation for this difference needs to be further investigated. The potency for 4-AcO DMT was 969.6 nM, whereas Klein et al. (2021) reported a potency of 100 nM. (67) The measured potency was in the higher nanomolar range, while the reported potency was in the middle nanomolar range. Again, there is a noticeable difference between the measured value for the potency and the value reported in literature. Further investigation is needed to explain this discrepancy.

4-AcO MPT had an efficacy of 77.36%. This efficacy showed that 4-AcO MPT was a partial agonist on the 5-HT_{2B}R. Comparing the measured efficacy with the efficacy reported in literature of 62.7%, there could be noticed that the obtained potency was higher than the reported potency, but the compound was still classified as a partial agonist on the 5-HT_{2B}R. (67) The potency for 4-AcO MPT was equal to 577.9 nM. Klein et al. (2021) reported a potency of 28.8 nM on the 5-HT_{2B}R. (67) The measured potency was in the higher nanomolar range while the reported potency was in the lower nanomolar range. Again, there could be noted that the measured values for the efficacy and potency were higher than the values reported in the literature. Possible explanations for the consistently higher values for both the efficacy and potency will be discussed further in this section.

Additionally, an analysis and comparison of the data with the literature for the 5 substituted tryptamines were performed. 5-MeO MiPT had an efficacy of 54.18% which showed that 5-MeO MiPT was a partial agonist on the 5-HT_{2B}R. Rickli et al. (2016) reported an efficacy of 12% which meant that the compound was classified as a weak partial agonist. (71) When comparing the measured efficacy with the reported efficacy of the compound, there could again be noted that the measured value was a higher than the reported value. The potency for 5-MeO MiPT was equal to 11.40 nM. Rickli et al. (2016) reported a potency of 1.5 µM, which was a value substantially higher than the observed potency. (71) The

obtained potency was thus not in line with the literature for this compound. In this experiment, 5-MeO MiPT seemed to have a better potency than previously reported.

5-MeO AMT had an efficacy of 72.75%. This showed that 5-MeO AMT was a strong partial agonist on the 5-HT_{2B}R. Rickli et al. (2016) reported an efficacy of 104%, which meant that the compound was classified as a full agonist on the 5-HT_{2B}R. (71) The obtained value for the efficacy was thus substantially lower than the reported value. The measured efficacy of 5-MeO AMT was not in line with the literature. The potency of 5-MeO AMT was equal to 15.58 nM. Rickli et al. (2016) reported a potency of 4 nM. (71) Both the measured and reported potency were in the lower nanomolar range. The potency of 5-MeO AMT was in line with the literature.

5-chloro tryptamine had an efficacy of 36.97% and a potency of 77.51 nM. Since there was no literature available on this compound, a comparison could not be made. The results for efficacy and potency obtained here could be trusted since the results for the internal check, 5-HT, were in line with literature. 5-chloro tryptamine was thus a partial agonist on the 5-HT_{2B}R and had a potency in the lower nanomolar range.

The efficacy and potency of LSD, 5-HT, and the tryptamine derivatives were also determined on the 5-HT_{2A}R. Since only 1 independent experiment for the 5-HT_{2A}R was performed, it would be premature to analyse and compare the results from this experiment with the literature. The results were underpowered, and more experiments should be performed to confirm or deny the reported numbers for efficacy and potency. No statistical analysis was done on the obtained data since the study was severely underpowered.

The data was normalized in function of % LSD, and the efficacy of LSD was again set to 100%. When looking at the potency of LSD determined by the EC_{50} value, LSD had a potency of 367.1 nM. Rickli et al. (2016) stated a potency of 261 nM. (71) Both the measured potency and reported potency were in the mid nanomolar range, thus the potency of LSD could be considered in line with literature even though the obtained value was higher.

The efficacy for 5-HT on the 5-HT_{2A}R was 106.8%. When looking at the literature, 5-HT was a full agonist on the 5-HT_{2A}R and a stronger agonist on the 5-HT_{2A}R than LSD. In these experiments, it seemed that 5-HT was a full agonist on the 5-HT_{2A}R. Therefore, the obtained efficacy of this compound was in line with literature. The obtained EC_{50} value for 5-HT was 413.4 nM. Klein et al (2021) stated a potency of 0.26 nM for 5-HT on the 5-HT_{2A}R, which was in the lower nanomolar range. The measured value was in the mid nanomolar range. When comparing the measured potency and the reported potency, both were in the nanomolar range, thus the potency for 5-HT could be considered in line with literature even though the obtained value was higher.

The same 5 tryptamine derivatives were tested. 4-AcO DMT seemed to be a partial agonist on the 5-HT_{2A}R with an efficacy of 86.19%. Klein et al. (2021) reported an efficacy of 79.2% stating that 4-AcO DMT was a partial agonist on the 5-HT_{2A}R. (67) The variability in efficacy was high. More experiments should decrease this variability. The obtained efficacy was also slightly higher than the reported efficacy. The potency for 4-AcO DMT was equal to 2.021 µM. Klein et al. (2021) reported a potency of 103 nM. (67) The measured potency was in the low micromolar range while the reported potency was in the low nanomolar range. There could be noted that the measured value for the potency was higher than the value reported in the literature.

4-AcO MPT had an efficacy of 11.46%. This efficacy showed that 4-AcO MPT was a weak partial agonist on the 5-HT_{2A}R. Klein et al. (2021) reported an efficacy of 96.0%, which meant that the compound was classified as a strong partial agonist or even a full agonist. (67) There could be noticed that the obtained efficacy was way lower than the reported efficacy. Therefore, the measured efficacy for 4-AcO MPT was not line with the literature. The potency for 4-AcO MPT was equal to 29.64 nM. Klein et al. (2021) reported a potency of 42.4 nM on the 5-HT_{2A}R. (67) Both the measured potency and the reported potency were in the lower nanomolar range. The potency of 4-AcO DMT was in line with the literature.

5-MeO MiPT had an efficacy of 159.7% which showed that 5-MeO MiPT was a full agonist on the 5-HT_{2A}R. Rickli et al. (2016) reported an efficacy of 83.0%, which meant that the compound was classified as a strong partial agonist. (71) The obtained efficacy

was higher than the reported efficacy. An explanation on the difference in efficacies is need and will be given further in this discussion. The potency for 5-MeO MiPT was equal to 263.5 nM. Rickli et al. (2016) reported a potency of 23 nM. (71) Both the measured and reported values for the potency were in the lower nanomolar range but the measured potency is higher. The obtained potency was in line with literature.

5-MeO AMT had an efficacy of 115.2%. This showed that 5-MeO AMT was a full agonist on the 5-HT_{2A}R. Rickli et al. (2016) reported an efficacy of 60% which meant that the compound was classified as a partial agonist on the 5-HT_{2A}R. (71) The obtained efficacy was higher than the reported efficacy. The potency of 5-MeO AMT was equal to 4.665 nM. Rickli et al. (2016) reported a potency of 2 nM. (71) Both the measured and reported potency were in the lower nanomolar range. The potency of 5-MeO AMT was in line with literature.

5-chloro tryptamine had an efficacy of 115.2% and a potency of 29.47 nM. Since there was no literature available on this compound, a comparison could not be made. 5-chloro tryptamine was thus a partial agonist on the 5-HT_{2A}R and had a potency in the lower nanomolar range.

As mentioned previously in this discussion, the efficacy for 4-AcO DMT, 4-AcO MPT, 5-MeO MiPT on the 5-HT_{2B}R were substantially higher than the values reported in literature. Also, the EC_{50} values that resemble the potency for 4-AcO DMT, 4-AcO MPT, and 5-MeO AMT on the 5-HT_{2B}R were higher than the values reported in the literature. The same trend was noticed for the efficacies of 4-AcO DMT, 5-MeO MiPT, and 5-MeO AMT on the 5-HT_{2A}R and the potencies of LSD, 5-HT, 4-AcO DMT, and 5-MeO MiPT on the 5-HT_{2A}R. An explanation for these higher values could be that in these experiments LSD was used as the reference agonist whereas in the articles describing the tryptamine derivatives, 5-HT was used as the reference agonist. 5-HT is a stronger agonist than LSD on the 5-HT_{2B}R and a much stronger agonist than LSD on the 5-HT_{2A}R. This was also shown in the performed experiments. When all data is normalized in function of % 5-HT instead of % LSD, it makes sense that the efficacy and potency reported in literature for the tryptamine derivatives were lower than the values that were obtained during these experiments.

A second possible explanation for the consistent higher values could be that for these experiments a different experimental method, the AequoScreen Assay, was used in comparison with the literature where the FLIPR assay was used. Both assays measured Ca^{2+} flux as a result of the activation of the 5-HT_{2B}R and the 5-HT_{2A}R. During the AequoScreen assay, cells undergo a lengthy preincubation period of 4 hours where coelenterazine h is able to interact with the apo-enzyme, apoaequorin. Upon binding, the active enzyme aequorin is formed. In the cytosol of the cells, a basal level of Ca^{2+} is present which can bind to aequorin. This reaction causes a change and increase in the luminescent signal of the cell. On the other hand, in the FLIPR assay the drugs are added to the cells followed by loading the cells with the fluorescent dye and an immediate measurement of the fluorescent signal.

The biggest difference between the two experimental methods is the lengthy 4-hour pre-incubation period for the AequoScreen assay in comparison with the immediate measurement of the fluorescent signal in the FLIPR assay. The lengthy pre-incubation period ensures that all the Ca^{2+} in the cell will bind the activated aequorin and increase the basal luminescent signal for every well, whereas the FLIPR assay does not have a high basal signal due to immediate measurement. In other words, every Ca^{2+} ion gets the opportunity to bind to the activated enzyme and increase luminescent signal in the AequoScreen assay, but not every Ca^{2+} ion in the cell gets the opportunity to influence the fluorescent signal in the FLIPR assay. This can explain the consistently higher values for the efficacy.

This hypothesis could be confirmed by performing the AequoScreen assay and FLIPR assay with a 5-HT_{2A}R and 5-HT_{2B}R antagonist, e.g., ketanserine and compare the results from both assays. The efficacy for ketanserine should be higher in the AequoScreen assay than the FLIPR assay due to a higher baseline signal in the AequoScreen Assay. Due to time constrains this experiment was not performed. The second explanation is thus solely a hypothesis that needs to be confirmed.

6 CONCLUSION

After testing the activity of LSD, 5-HT, and the tryptamine derivatives on the 5-HT_{2B}R and 5-HT_{2A}R, the following conclusions could be made. The values reported in this dissertation might deviate (i.e., higher) from those reported in the literature. These deviations are accepted here because, in these experiments, the data was normalized in function of % LSD instead of % 5-HT. Additionally, a different experimental method, the AequoScreen assay, was used instead of the FLIPR assay.

4-AcO DMT was a partial agonist on the 5-HT_{2B}R and a strong (>80%) partial agonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and the 5-HT_{2A}R was 969.9 nM and 2021 nM respectively. 4-AcO MPT was a partial agonist on the 5-HT_{2B}R and a weak (<20%) partial agonist or even antagonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and 5-HT_{2A}R was 577.9 nM and 29.64 nM. 5-MeO MiPT was a partial agonist on the 5-HT_{2B}R and a full agonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and 5-HT_{2A}R was 11.40 nM and 263.5 nM. 5-MeO AMT was a partial agonist on the 5-HT_{2B}R and a full agonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and 5-HT_{2A}R was 15.58 nM and 4.665 nM. 5-chloro tryptamine was a partial agonist on the 5-HT_{2B}R and a full agonist on the 5-HT_{2A}R. The potency on the 5-HT_{2B}R and 5-HT_{2A}R was 11.40 nM and 29.47 nM.

After testing the activity of all the tryptamine derivatives on the 5-HT_{2B}R and the 5-HT_{2A}R, it is evident that each compound is at least active on the 5-HT_{2B}R, on the 5-HT_{2A}R or even both receptors. A one-way ANOVA test, followed by a post-hoc Bonferroni's test showed that there were no statistically significant differences between the efficacy and potency of LSD and the tryptamines derivatives on the 5-HT_{2B}R. All tryptamine derivatives are thus able to substantially activate the 5-HT_{2B}R just like LSD. No statistical analysis for the compounds on the 5-HT_{2A}R could been done since an insufficient number of experiments were performed.

All tested tryptamine derivatives should be considered at least equally as dangerous as LSD by Health Canada when freely available on the illicit market. To decrease the danger of these compounds and since LSD is already scheduled, the tryptamine derivatives should also be scheduled. Considering that LSD (and its salt derivatives) and

DMT (and its salt derivatives) are scheduled in schedule III of the Controlled Drugs and Substances Act, it would make sense for these tryptamine derivatives to be scheduled in schedule III as well. (90)

This data shows once again that the scheduling system used in Canada is not a reliable system. By making a small modification to a scheduled drug, the modified drug is no longer considered illegal. This system asks for continuous monitoring of the drug market so that the schedules can be updated regularly. It would be easier and safer if the scheduling system in Canada would be replaced by a legal system equivalent to the one in Belgium where substance groups (e.g., the tryptamines) are regulated.

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